

The untreated group (Table II) was fed this corn with a supplement of tankage and bone meal. Pigs 492 and 496 died and were immediately autopsied. Typical myelin sheath degeneration was observed. Pigs 487 and 510 were killed to obtain fresh tissues. These also presented degenerated myelin sheaths. Pigs 504, 511 and 512 were placed on a ration that was high in vitamin A and its precursors. In these pigs the symptoms of incoordination disappeared at the end of about 3 months. They were again able to stand and walk (Figs. 1 and 2). The symptoms could be brought back by severe exertion and fatigue but would disappear after rest. The recovered animals were kept on the ration high in vitamin A for an additional month, after which time Pigs 511 and 512 were killed. Severe myelin degeneration was found in these two animals although the clinical symptoms had disappeared (Fig. 3). The factor that corrected the incoordination apparently had no appreciable effect on the myelin degeneration. At this time Pig 504 was placed on a vitamin A-deficient ration for nearly 5 months for the purpose of bringing back the symptoms of incoordination, but without success. On postmortem examination the myelin degeneration was not quite as severe as in Pigs 511 and 512.

The authors are indebted to Prof. V. E. Nelson, of the Department of Chemistry, for assay of the corn from the field cases cited in Table II. The results were not available until the close of the experiment. It showed that the vitamin A content did not differ from that of the corn used on the Institute farm where incoordination appeared only in experimentally produced cases. A consideration of the evidence in this and subsequent experiments in which we were unable to cure the condition by cod liver oil alone suggests that some factor other than vitamin A corrected the incoordination.

A ration known to be deficient in vitamin A, as ascertained by assay on chicks, was fed to a group of pigs from farrowing time until the animals were killed for pathological material. In all cases myelin degeneration was present (Table III). Pig 569 with complete inability to stand or walk on its hind legs was brought indoors and given massive doses of cod liver oil and a vitamin B concentrate. No improvement was noted in 2 months so it was killed. Controls 522, 531 and 532 were fed the same ration as the others but as soon as they developed a stilted gait at 4 months of age yellow corn was substituted for white corn and cod liver oil added to

the diet. The objective was to check and cure the condition in its early stage.

The severity of the incoordination was not correlated with that of

TABLE III  
*Data on Swine Fed Diet Deficient in Vitamin A*

BASAL DIET

White corn .....	78%
Tankage .....	20%
Bone meal .....	2%

Pig No.	Time on diet	Incoordination	Myelin degeneration		
			Sciatic	Brachial	Cord
	<i>mos.</i>				
523	9	Slight	+		+
524	10	"	++	+	+
527	12	"	+		+
528	12	"	+	+	+
529	12	"	+	+	+
530	12	"	+	+	+
534	14	"	T	T	+
535	14	"	T	T	+
555	7½	"	T	T	+
552	8	Complete	T	T	+
*569	9½	"	+	+	+
574	9	None	T	T	+

*Controls*

BASAL DIET

Yellow corn .....	78%
Tankage .....	20%
Bone meal .....	2%
Cod liver oil .....	15 cc. per pig daily

522	9	Stilted gait	+		+
532	12	" "	+	+	+
531	12	" "	+	+	+

\* Cod liver oil and Harris B concentrate — no effect.  
T = Trace.

the myelin degeneration. It is also pointed out that these rations contained adequate vitamin B complex. Tests on day old chicks showed that corn supplies sufficient vitamin B complex when fed at a level of 75 per cent of the ration.



The storage of vitamin A was next considered in a group of swine. Sows were placed on white corn, skim milk powder and bone meal from 1 to 3 months before breeding. Both white corn and the skim milk powder had been assayed on chicks and found to be very low in vitamin A but adequate in vitamin B<sub>1</sub>. The pigs of this group (Table IV) developed symptoms of incoordination very early in life,

TABLE IV

*Data on Pigs from Sows Fed 1-3 Months Prior to Breeding on 79% White Corn, 20% Skim Milk and 1% Bone Meal*

Pig No.	Age <i>mos.</i>	Incoordination	Myelin degeneration		
			Sciatic	Brachial	Cord
655	3	Advanced	T	T	+ D
654	3	"	T	T	+ D
656	3	"	T	T	D
646	4	"	T	T	D
647	4	"	T	T	+ K
657	7	"	T	T	+ K

*Skim Milk Powder ad lib.*

671	8	Recovered	T	T	T K
665	10	"	T	T	+ K
743	12	"	?	?	? K
744	12	"	?	?	? K

*Controls Received Yellow Corn + Cod Liver Oil After Early Symptoms*

748	13	None	T	T	+ K
749	13	"	T	T	+ K

T = Trace.  
D = Died.  
K = Killed.

before weaning in many instances. The severity of the incoordination increased rapidly in this group, some individuals succumbing as early as 3 months of age. These pigs, unlike those shown in Table III, showed severe incoordination but only mild nerve degeneration and less severe changes in the cord. Pigs 671, 665, 743 and 744 were given skim milk powder *ad lib.* at the age of 4 months. After 3 months the ration was changed to include equal amounts of ground

white cor. and skim milk powder. Pigs 748 and 749 were fed yellow corn and cod liver oil at  $2\frac{1}{2}$  months of age.

Pigs 743 and 744 (Table IV) fed large amounts of skim milk powder until 12 months of age revealed no changes in the myelin sheaths by means of the Marchi method. The fat adjacent to the nerves also failed to react. A failure in technic was considered. The blocks were blackened but when microtome sections were made these appeared yellowish gray. The remaining portion of the Müller's fluid-osmic acid mixture was used on control material which reacted in the conventional manner by blackening fat and degenerated myelin. Investigations are underway to determine the presence of some factor in skim milk powder that may be responsible for this behavior. It should also be noted that only traces of degeneration could be demonstrated in Pigs 671 and 665 fed large quantities of skim milk powder for 8 and 10 months respectively.

In Figures 4, 5 and 6 are shown cords of pigs showing degeneration on low vitamin A rations, adequate vitamin A rations and a normal control.

Grains are known to contain vitamin B but since many of them are stored long periods and often harvested in an immature state the possibility of a vitamin B deficiency as the cause of demyelination of the nerves of swine was considered.

The presence of myelin degeneration after recovery from incoordination (Table II) and the findings in rats by Zimmerman suggested the need of some data for comparative purposes on the effect of vitamin B<sub>1</sub>-free rations on swine. In attempting to evaluate a change such as myelin degeneration it should be realized that basal rations used in nutritional studies are extremely limited and artificial. In an effort to control a given vitamin we believe unknown factors are introduced.

Normal swine of various sizes were fed diets previously assayed on chicks for vitamin B<sub>1</sub>. Any rations fed chicks, 24 hours after hatching, which died of polyneuritis within 7 to 12 days, were considered deficient in B<sub>1</sub>. In Table V are shown the results of the effect of the presence or absence of B<sub>1</sub> on myelin degeneration of the nerves of swine on restricted diets. The type of degeneration is identical with that shown in Figures 1 and 2. These results show definitely that vitamin B<sub>1</sub> does not play a part in the production of these lesions since both the controls and the B deficient animals show degenera-

tion. Since cod liver oil was fed to all pigs on the B<sub>1</sub> experiment, vitamin A was eliminated as a causal factor.

Evidence obtained from other species has indicated that diets lacking in some unknown factor, exclusive of vitamins A and B<sub>1</sub>, will produce myelin degeneration. Biester, Greenwood and Nelson,<sup>11</sup>

TABLE V

*Vitamin B<sub>1</sub>-Free Rations Fed Swine\**

Polished rice + washed casein 10%, tankage 10%  
 Polished rice + tankage 20%  
 Polished rice + washed casein 10-20%\*\*

TISSUES	INCOORDINATION	MYELIN DEGENERATION
No. pigs 7	None	Spinal cord 100%

*Vitamin B<sub>1</sub>-Containing Rations\**

Polished rice + untreated skim milk powder 10%, tankage 10%  
 Polished rice + tankage 20% + yeast 2.1%  
 Polished rice + washed casein + yeast 2.1%

TISSUES	INCOORDINATION	MYELIN DEGENERATION
No. pigs 5	None	Spinal cord 100%

\* Each basal ration included bone meal 1%, NaCl 0.3%, and 15 cc. cod liver oil per pig. FeCl<sub>3</sub> was fed to groups not receiving tankage.

\*\* 10% fed during first 104 days.

using dogs during the course of studies on fluorine toxicology, found severe myelin degeneration in the spinal cords of both fluorine-fed and control animals (Fig. 7).

During the first 4 months the dogs received 180 cc. of whole milk and a commercial dog preparation containing 19 per cent digestible protein. Vitamins A, B, D and G were amply supplied. The vitamin C content of the ration was low. From 4 months of age until destruction the dogs received a daily ration patterned after that of Mellanby, which consisted of the following:

Yellow corn .....	30 parts
Hulled oats .....	30 "
Ideal dog food (commercial) .....	23 "
Wheat germ meal .....	5 "
Skim milk powder .....	10 "
Cod liver oil (Squibb) .....	1 "
Sodium chloride (0.01 gm. NaI per 100 gm.) .....	1 "
and	
Whole milk .....	180 cc.

The yellow corn and hulled oats were moistened, autoclaved  $1\frac{1}{2}$  hours at 15 pounds pressure and then dried. This was done to render them more palatable before incorporation in the mixture. The commercial dog food contained approximately 12 per cent protein. This ration included sufficient quantities of vitamins A, B, D and G.

The dogs fed the above ration showed, in addition to the spinal cord lesions, fatty degeneration of the renal epithelium in the medullary rays confined chiefly to the spiral convoluted tubules. This characteristic distribution could be seen plainly without magnification in the slides prepared by the Marchi method. These dogs manifested no incoordination or other clinical symptoms. Blood and urine examinations by standard chemical methods were negative for nephritis.

Day old chicks were kept on the same rations as the swine. Although the chicks succumbed or were moribund as a result of A or B<sub>1</sub> deficiencies respectively, myelin degeneration in the sciatic nerves was absent in most cases while in a few animals a few fibers were affected. The absence of or relatively slight nerve lesions found in these chicks was ascribed to the short duration of the experiments. This may also account for the results of Campos, Campos and Maffei,<sup>10</sup> who fed rats vitamin-deficient rations for relatively short periods of time. The high protein rations (42 per cent casein) used by these authors may be a factor, since unconfirmed results obtained in one of our experiments indicate that *ad lib.* feeding of skim milk powder will cure incoordination and produce a regeneration of the myelin sheaths (Pigs 671, 665, 743, 744 (Table IV)). Further experimentation on this point is underway. Field cases of incoordination were cured by *ad lib.* feeding of skim milk powder. Unfortunately tissues from these cases were not available.

#### SUMMARY AND CONCLUSIONS

These experiments suggest that the incoordination and myelin degeneration in the nervous systems of swine are caused by different etiological agents.

Severe myelin degeneration has been produced in the absence of incoordination.

Experiments conducted over a period of 4 years have shown that

neither vitamins A nor B complex are responsible for the myelin degeneration of the spinal cords and peripheral nerves in swine.

Severe myelin degeneration without incoordination was found in dogs fed a ration that included vitamins A, B complex, D and E.

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## DESCRIPTION OF PLATE

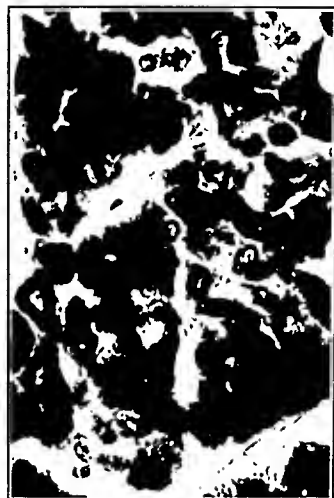
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### PLATE 47

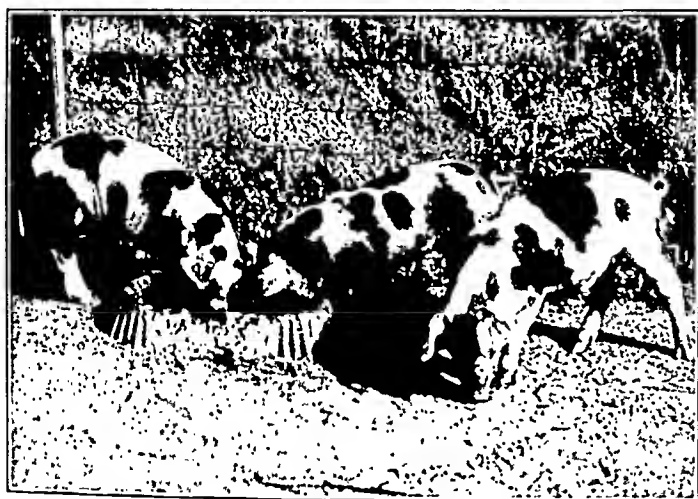
- FIG. 1. Showing affected pigs (Nos. 511, 512 and 504).
- FIG. 2. Same pigs 3 months after change of ration.
- FIG. 3. Spinal cord from recovered case, Pig. 511. Marchi method.  $\times 400$ .
- FIG. 4. Spinal cord from pig receiving ration containing B complex but deficient in A. Marchi method.  $\times 400$ .
- FIG. 5. Spinal cord from pig receiving adequate A and B complex. Marchi method.  $\times 400$ .
- FIG. 6. Spinal cord from control pig kept on pasture. Marchi method.  $\times 400$ .
- FIG. 7. Spinal cord from dog receiving vitamins A, B complex, D and E. Marchi method.  $\times 400$ .



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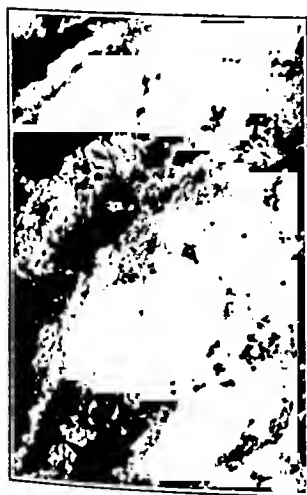
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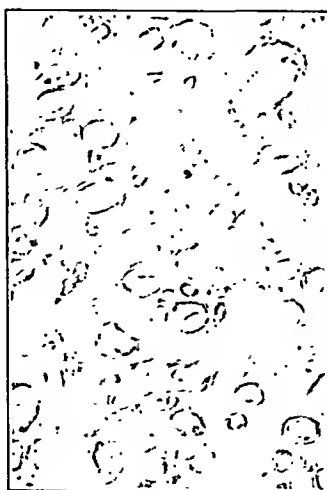
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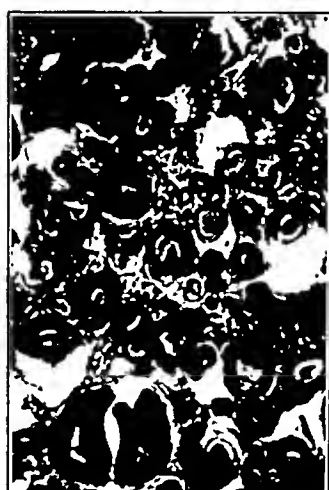
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5



6



7

Eveleth and Biester

Myelin Sheath Degeneration





## MESENTERIC CHYLADENECTASIS\*

### REPORT OF A CASE

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Although moderate dilatation of lymph node sinuses is commonly seen, extensive dilatation with the formation of multiloculated or single cysts is exceedingly rare. Ectasia of the mesenteric lymph nodes with inspissation of chyle is apparently even more unusual. Von Rokitsky<sup>1</sup> first discussed this condition in 1855. He reported a case in which multiloculated dilatation of the mesenteric lymph nodes was found. Fatty material filled these cystic spaces.

Gjorgjević<sup>2</sup> in 1871 first proposed the term lymphadenectasis to describe dilatation of lymph nodes. Odenius<sup>3</sup> classified cystic lymphangioma separately from simple dilatation of lymph node sinuses. This latter condition was termed lymphadenocoele, while development of a central cyst by gradual distention was called simple cystic metamorphosis.

Many theories to explain the dilatation of lymph nodes and lymphatics have been proposed. The mechanical theory suggested by von Rokitsky<sup>1</sup> and Killian<sup>4</sup> emphasized passive formation of dilatation due to obstruction of lymph vessels. Klebs<sup>5</sup> mentioned increased lymph production peripherally. Obstruction of the thoracic duct in the etiology has been advocated by Enzmann,<sup>6</sup> Killian,<sup>4</sup> and Virchow.<sup>7</sup> So-called infarction of efferent vessels caused by inspissated chylé has been suggested by von Rokitsky<sup>1</sup> and Spaeth.<sup>8</sup> Gross<sup>9</sup> produced congestion of chyle by tying the thoracic duct and the iliac vein. He believed that a congestion of circulation was also necessary. Naumann<sup>10</sup> spoke of a chronic desquamative lymphangitis as a possible cause of mesenteric cysts. Orth<sup>11</sup> attributed local disturbances of lymph circulation to inflammatory changes.

The dilatation of lymph channels and chylous retention cysts has even been linked with neoplasm. In the cases of lymphangioma and chyliangioma, Wegner<sup>12</sup> and Sudhoff<sup>13</sup> have assumed that first re-

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tention congestion may occur, followed by irritation or by trauma, and the result is the formation of a neoplasm, chylangioma or lymphangioma. Sudhoff<sup>13</sup> went even farther and assumed that both retention cysts and true chylangioma might occur in the same patient simultaneously. However, Swartley<sup>14</sup> believed that cystic malignant disease of the mesentery, as suggested in the classification of Carter,<sup>15</sup> was not tenable. He assumed malignant changes occurred in already existing cysts.

The case herein reported does not fall in the group of true neoplasms or the classification of mesenteric cysts arising from embryonic rests emphasized by Dowd,<sup>16</sup> Ney and Wilkinson,<sup>17</sup> and Swartley.<sup>14</sup> The cystic changes of the lymph nodes more closely simulated the lesions originally reported by von Rokitansky.<sup>1</sup>

Clinically this case is of interest because of the difficulty of diagnosis. The outstanding symptoms of ravenous appetite, somnolence and emaciation were also noted by Wilson<sup>18</sup> in his report of a large chylous cyst.

#### REPORT OF CASE

*Clinical History:* The patient, a white Polish farmer, aged 60 years, was admitted to the hospital with the complaint of vomiting, diarrhea, and pain in the epigastrium for 2 months. No history of any relative past illnesses could be obtained with the exception of a similar attack 1 year previous. The latter assertion was made on only one occasion and could not be confirmed.

Physical examination revealed an extremely emaciated white male, mentally confused. The only positive findings consisted of oral sepsis, a few râles heard over the base of the lungs and poor heart tones. Palpation of the abdomen and rectal examination revealed nothing. The blood pressure was 110/70. The hemoglobin was 60 per cent by Sahli, the red cell count 3,250,000, the white cell count 6800, of which 73 per cent were polymorphonuclears and 27 per cent lymphocytes. The blood chemistry showed a blood sugar of 35 mg. after the specimen had been in the icebox overnight. The chemistry was otherwise negative. X-ray study of chest and gastro-intestinal tract was not diagnostic.

The patient's condition became progressively worse, although he made no definite complaints. He became extremely weak and hypotension developed. His appetite was good but he was somnolent.

The temperature never exceeded normal, except during the period just before death, and was for the most part subnormal, around 97° F. The pulse ranged from 70 to 80.

Mental confusion developed, then coma, and the patient died 7 weeks after entering the hospital.

#### POSTMORTEM EXAMINATION

The body was that of an extremely emaciated white male, 60 years of age, 165 cm. in length, weighing 90 pounds. The skin was pale.

*Thorax:* There were about 200 cc. of straw colored fluid containing small flakes and strings of fibrin in the left pleural space. The lungs were intensely edematous with passive hyperemia posteriorly. There were also poorly outlined, irregular areas of slightly increased density in these congested portions of the lungs. The heart showed adherence of the two layers of the pericardium. The myocardium was a dark brown color, extremely flaccid with a peculiar gelatinous appearance and almost diffuent in consistence.

*Abdomen:* The liver and spleen showed moderate chronic passive congestion. The gastro-intestinal tract was edematous and congested throughout. The pancreas exhibited an increase in density, and a moderate nephrosclerosis of both kidneys was found.

*Mesentery:* All the mesenteric lymph nodes were enlarged, and ranged in size from 0.8 to 3 cm. in diameter. They were firm, smooth, discreet, and on section all exhibited a cystic spongy appearance. The same characteristics were apparent in even the smallest mesenteric lymph nodes. The cut surface was of a cream white color with a fatty material exuding from the small cystic spaces. This same type of lymph node enlargement was likewise found retroperitoneally around the pancreas.

The head and neck were not dissected.

*Anatomical Diagnoses:* Mesenteric chyladenectasis, chronic and acute mesenteric and retroperitoneal lymphadenitis and lymphangitis, chronic pancreatitis with islet hyperplasia, moderate generalized arteriosclerosis, nephrosclerosis, brown atrophy of heart, chronic passive hyperemia of liver, spleen, and gastro-intestinal tract, chronic fibrous adhesive pericarditis, hypostatic bronchopneumonia, and acute serofibrinous pleuritis.

### HISTOLOGICAL FINDINGS

On microscopic examination the spongy cystic structure of the lymph node resolved itself into dilated, tortuous communicating spaces, entirely filled with homogeneous lipoid material (Fig. 1). The dilated spaces had no consistent cell lining, but instead were limited by the reticulum fibers and cells of the lymph node stroma. However, there were fairly frequent giant cells which were occasionally sufficiently numerous to give the semblance of a complete lining. These cells appeared actively phagocytic with numerous fine particles of fat in their cytoplasm, and not infrequently engulfed

entire leukocytes. In structure the giant cells were of the usual foreign body type with somewhat oval shaped nuclei having no characteristic arrangement.

The lymph node stroma consisted of the usual reticulum showing numerous bands of thickening and areas of fibrosis. Thickening of the capsule was likewise found in all the involved lymph nodes. Not infrequently areas of hyalinization of the reticulum were encountered. This process was most common in the group of lymph nodes adjacent to the pancreas. In these, too, the lymph nodules often exhibited central hyalinization, and in some instances complete obliteration of the nodules resulted.

The cellular elements of the lymph nodes were interesting because of their variety. In addition to the giant cells already noted there was an increase in the number of the reticulum cells, likewise most notable in the areas close to the dilated sinuses (Fig. 2). Various stages of differentiation from the small simple type with oval, chromatin-poor nuclei and scanty, somewhat elongated, poorly defined cytoplasm, all the way up to the giant cell were found. The intermediate forms exhibited widening of the cytoplasm with definite cytoplasmic boundaries and a slightly larger round nucleus with occasional prominent nucleoli. These more differentiated forms of reticulum cells contained many fine fat droplets in their cytoplasm. In addition fairly numerous plasma cells and tissue mast cells were demonstrated. The lymphocytes for the most part were arranged diffusely and in small, poorly outlined collections. This breaking up of the lymph nodule architecture was, of course, more pronounced in the nodes showing the greatest degree of ectasia.

Scattered polymorphonuclear neutrophils were found in all lymph nodes but were relatively scarce in those of the lower part of the mesentery, and much more numerous in the lymph nodes in the pancreatic group. In the latter instance actual plugging of the lymphatic vessels and parts of the cortical sinuses with leukocytes could be seen.

A search for microorganisms demonstrated the presence of Gram-positive cocci in chains in the lymphatic vessels and lymph nodes. Their number ran parallel with the degree of polymorphonuclear leukocytic infiltration. In the lower mesenteric nodes small numbers of these organisms were found, most frequently located in the perivascular lymph spaces.

Examination of the sinus contents showed a homogeneous material which took a uniform red stain with scharlach R. Polariscopic study did not reveal anisotropic characteristics.

No increase of smooth muscle fibers was found in these lymph nodes. The dilated sinuses particularly failed to exhibit an indication of smooth muscle as found in lymph vessel walls.

The loose fibrous tissue of the mesentery adjacent to lymph nodes showed diffusely scattered plasma cells and large numbers of dilated lymph capillaries. The capillaries were made up of flattened endothelial cells only. No mitoses or atypical cytological features were seen. These minute vessels were completely filled with lipid material identical with that described in the lymph nodes.

*Pancreas:* There was definite increase of the interstitial fibrous tissue, particularly of that around the duct system, with the frequent formation of a partially hyalinized fibrous tissue collar. Polymorphonuclear neutrophils could be demonstrated in the periductal lymphatics and were found entirely occluding the large lymphatics in the peripheral portions of the pancreas. An unusual feature was the proliferation of the intercalary ducts and the centroacinar cells and an increase in number of large islands. In a few areas where exocrine glandular epithelium was almost entirely replaced by fibrous tissue, a proliferation of islet cells assumed the proportion of an adenomatous type of hyperplasia. Scharlach R preparations showed increased lipid content in the islet cells, sharply differentiating them from the surrounding parenchyma.

*Intestine:* A definite filling with fat and some distention of the lacteals and associated lymphatics was demonstrated in scharlach R preparations.

Microscopic examination of other organs showed hypostatic bronchopneumonia, moderate nephrosclerosis, and brown atrophy of the heart.

## DISCUSSION

The lymph node lesions in this case appeared to be due to dilatation by chyle, with subsequent breakdown of the emulsion and inspissation. The causative obstruction must have been of a widespread and diffuse character, in view of the rich collaterals in the lymphatic drainage of the mesentery. Such a requirement was provided by the unusual chronic inflammation and fibrosis in the

lymph nodes of the entire mesenteric, peripancreatic and upper retroperitoneal regions.

The attack of abdominal pain 1 year previous to hospitalization may have had a casual relation to the old hyalinized fibrosis of pancreas and lymph nodes. Obliteration of some lymphatic channels probably occurred. However, the more recent active chronic inflammation most likely accounted for the obstruction of the major portion of the lymphatic routes of drainage with consequent sinus dilatation. The possibility that this lymphadenitis in itself permitted the lymph nodes to dilate more readily should also be considered. The acute exudative inflammation of the lymphatics was apparently a terminal process with chylous stasis favoring the rapid spread of the infection.

As to the location and nature of the initial infectious process, one can only speculate. Both the pancreas and the intestine must be considered. Inflammatory changes were more prominent in the proximal group of lymph nodes in the pancreatic region while ectasia was the most evident feature of the mesenteric group. Nevertheless, some attempt at collateral compensation was seen in the presence of small amounts of fat in the peripancreatic lymph node sinuses and lymph vessels.

While somewhat similar gross and histological pictures are found in undoubted true neoplasms, chylangiomas or lymphangiomas, as described by Sudhoff<sup>13</sup> and von Haberer,<sup>19</sup> the case herein reported differed in several important features. The cystic spaces had all the characteristics of lymph node medullary sinuses, were definitely located in lymph nodes and had no true endothelial lining. Furthermore, smooth muscle could not be demonstrated around these cystic channels, as reported by Schmidt,<sup>20</sup> and von Haberer.<sup>19</sup> In addition the age of 60 years is against congenital factors, as in chylangiomas, which are reported most frequently in children and occasionally in young adults. Such cases have been reported by Royster,<sup>21</sup> Sudhoff,<sup>13</sup> Flynn,<sup>22</sup> Lauterburg,<sup>23</sup> Collins and Berdez<sup>24</sup> (Case 1), Harbitz,<sup>25</sup> and Ebhardt.<sup>26</sup> Finally, although there was some dilatation of intestinal lacteals and lymphatics, the typical cystic masses did not involve the intestine at any point.

The addition of chyladenectasis as a separate group to the classification of mesenteric cysts seems justified. Cases of lymph node dilatation resulting from inflammatory and mechanical factors are

probably not of the extreme degree of rarity indicated by a survey of the literature. Some have undoubtedly been reported as chylous mesenteric cysts and some probably as chyliangioma or cystic lymphangioma. The second case of Collins and Berdez<sup>24</sup> seems to be one of dilated lymph nodes.

The diagnosis of chyladenectasis must be considered in cases of multiloculated multiple cysts located in the mesentery and not involving the intestine wall, particularly in older adults. The finding of chylous or fatty contents in the dilated lymph node sinuses without a complete endothelial lining, the absence of smooth muscle fibers in the walls of the cystic spaces, and a suitable obstruction to chylous drainage are the main points in diagnosis.

### SUMMARY AND CONCLUSION

A case of mesenteric chyladenectasis is reported.

Microscopic study demonstrated lymph node sinuses dilated by inspissated chyle.

All the unusual cellular elements of lymph nodes were either inflammatory infiltrates, or reactive proliferations, attempting to remove the lipid material in the sinuses.

The case is reported because it seems to explain the genesis of one type of mesenteric cysts.

NOTE: The author is indebted to Dr. W. F. Jacobs, Buffalo City Hospital, for permission to report this case.

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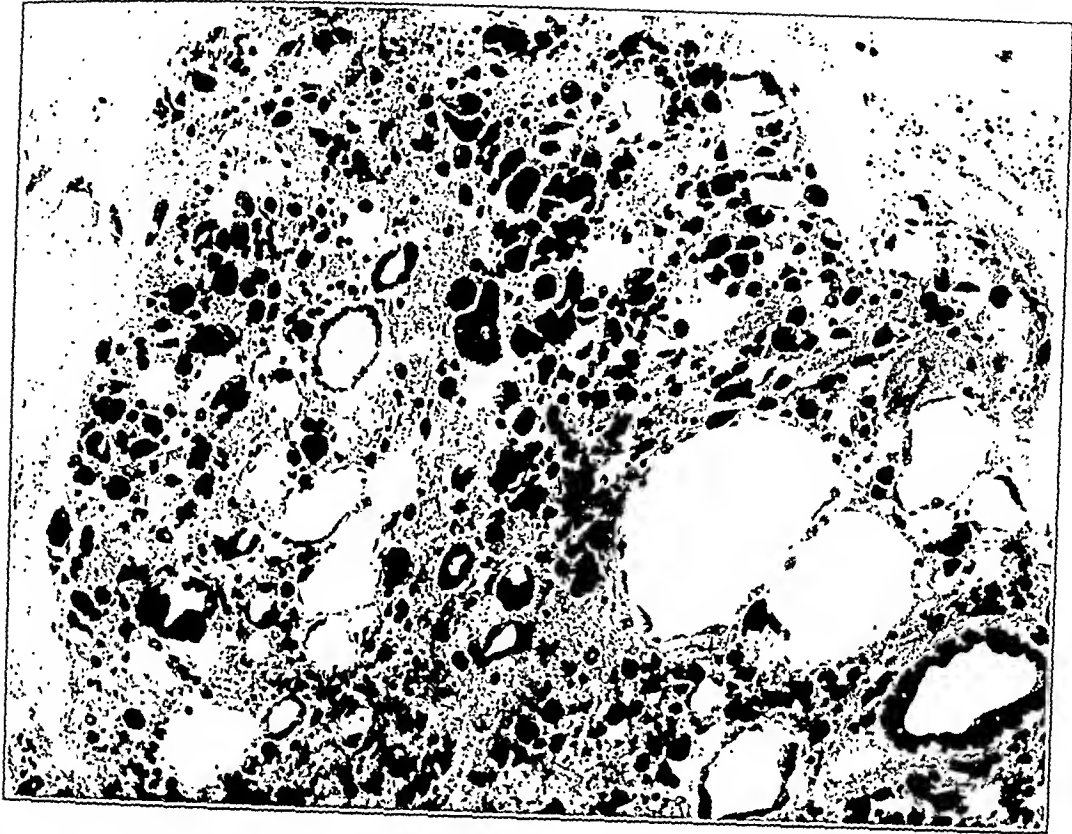
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## DESCRIPTION OF PLATE

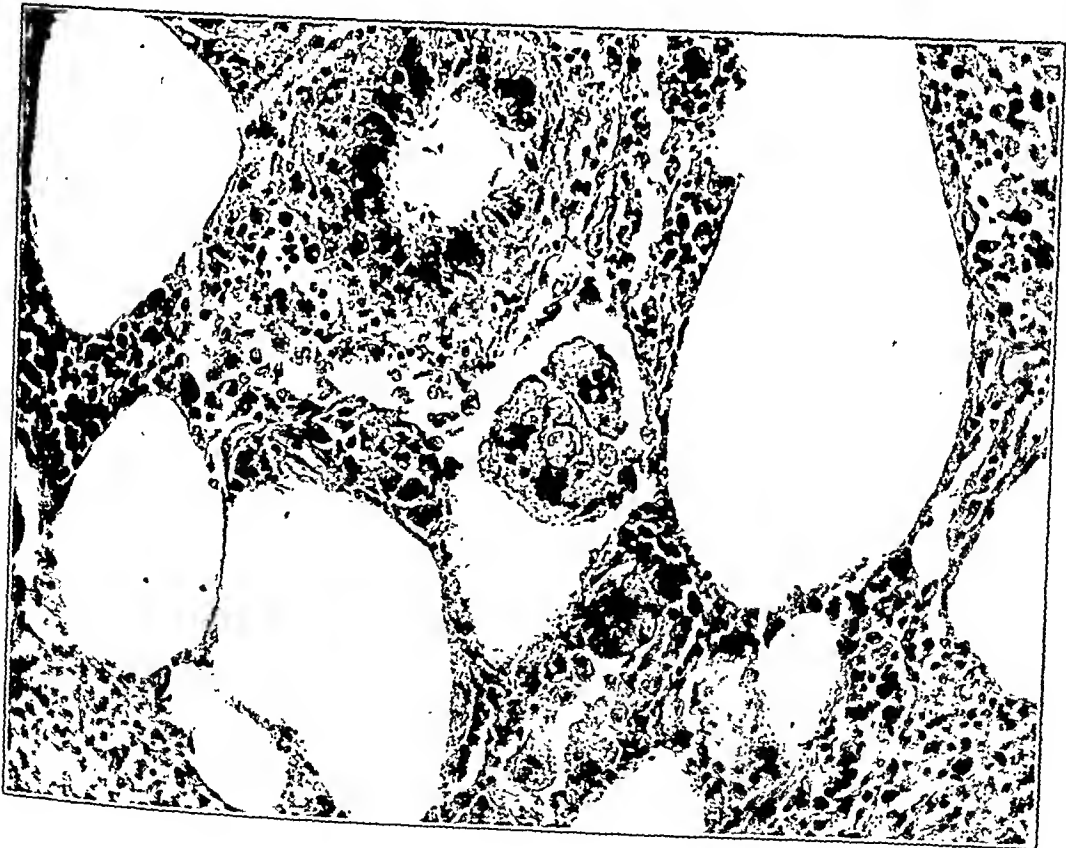
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### PLATE 48

- FIG. 1. Scharlach R stain showing dilated sinuses filled with lipoid material. The contents have fallen out of a few of the larger spaces during staining.  $\times 15$ .
- FIG. 2. Hematoxylin and eosin stain of a lymph node showing proliferation of reticulum cells and formation of giant cells.  $\times 250$ .



1



2

Hill

Mesenteric Chyladenectasis



## DIFFUSE PARIETAL ENDOCARDIAL SCLEROSIS \*

### REVIEW OF THE LITERATURE AND REPORT OF TWO CASES

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Acute and chronic valvular endocarditis have received considerable attention in the literature, but there are only a few reports of diffuse parietal endocardial sclerosis to be found. Moreover, most of the reports are somewhat confusing since there are included discussions of endocardial pockets (Zahn), which as isolated findings are to be differentiated, at least macroscopically, from the striking picture of the diffuse mural endocarditis under discussion. It is the purpose of this report to review the literature and to present 2 cases in which unusual pathological changes were found in the myocardium.

### REVIEW OF LITERATURE

To clarify the ensuing discussion it seems wise to describe first the architecture of the normal parietal endocardium. Nagayo,<sup>1</sup> after a thorough histological study, concluded that it consisted of five layers. These are, from the surface toward the myocardium, in the following order: (1) endothelial and subendothelial layer; (2) inner connective tissue layer; (3) elastic lamina; (4) smooth muscle layer; and (5) outer vascular connective tissue layer with the fibers of the Purkinje system.

It is evident from the variety of opinions expressed in the literature that there is no established etiological basis for this type of endocarditis. There is, however, a certain amount of agreement that the endocardial fibrosis in a group of these cases is functional in nature and is characterized histologically by the laying down of elastic fibers in a more or less orderly fashion, parallel to the surface, in the inner connective tissue layer of the endocardium.

Böger<sup>2</sup> believes that this group is characterized macroscopically, with only rare exceptions, by a diffuse, uniform grayish white thickening of the parietal endocardium, particularly in the left

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ventricle. Using a teleological argument, he concluded that a compensatory response of the parietal endocardium to prolonged dilatation is the cause of the sclerosis in this group. Hertel<sup>3</sup> makes no gross differentiation and from her material decided that the functional type was a result of prolonged hypertension or chronic myocardial disease. Karsner,<sup>4</sup> writing on sclerosis of the mural endocardium, particularly near the aortic orifice, believes that the fibrosis is essentially the same as intimal sclerosis with a possible inflammatory component. Nagayo<sup>1</sup> and Herxheimer<sup>5</sup> regard the parietal fibrosis seen in aged individuals, and particularly those with aortic valvular incompetence, to be the result of a chronic mechanical irritation by friction of the blood stream.

In the non-functional group the cause of the endocardial fibrosis has been given as contact or direct extension of the inflammation from valvular disease (Böger, Hertel, Dewitzky<sup>6</sup>), thrombosis of the vessels in the region of the endocardium (Nagayo, Hertel), extension from a myocarditis (Hertel, Dewitzky), and primary inflammation of the mural endocardium (Hertel, Dewitzky). Aschoff<sup>7</sup> believes that the majority of the cases without valvular disease are the result either of a parietal thromboendocarditis or of an atypical form of endocardial inflammation.

Mention must be made of the circumscribed elevated plaques that were a striking feature of the 2 cases reported here. One gathers from the literature that these plaques can occur with or without diffuse fibrosis and may or may not show pocket formation. It is difficult to decide from the reports and discussions whether or not isolated plaques are to be considered an entirely different pathological entity from those associated with more or less diffuse mural fibrosis. There is one point which may be of differential importance and that is that some of these plaques are described as light yellow rather than grayish white. Böger believes that plaque formation is a differential feature and commonly inflammatory, but rarely functional in origin. Herxheimer, on the other hand, concludes these plaques are of a functional nature. Dewitzky, histologically, differentiates two general groups, functional and inflammatory; the latter either primary on the endocardium or secondary by extension from valvular or myocardial disease.

The impression obtained from the literature is that two general groups of parietal endocardial fibrosis exist; one functional, the

other inflammatory. There is, however, little agreement as to the group in which many of the reported cases belong. This is due partly to the variety of histological and gross findings observed in this condition and partly to the different standards that are taken for classification.

### MATERIAL

In Case 1 it was possible to examine only the heart. Other information was obtained from the routine protocol. Blocks of tissue including endocardium and myocardium were taken from the larger fibrous plaques and other portions of both ventricles. Sections were also made from both auricles and the aorta. In Case 2 the heart was desired as a museum specimen and material was taken from the cut surfaces of both ventricles. The larger plaques were not investigated but smaller plaques were included in the material studied. The auricles and aorta, as well as the nodules on the mitral valve, were investigated. A large number of sections were made from each block. Hematoxylin and eosin, hematoxylin and Sudan III, and a combination of Weigert's elastic and Van Gieson's connective tissue stains were used.

### CASE REPORTS

**CASE 1. *Clinical Summary:*** The patient was a 41 year old male who had always been in good health and active athletically. His illness began a year before death and was characterized by a gradually increasing number of fainting spells which developed into epileptiform attacks, and the progressive development of non-valvular cardiac failure with periods of bradycardia. There was no past history or clinical evidence of infection. The Wassermann reaction was negative. Repeated electrocardiogram tracings showed either a right or left bundle-branch block or complete heart block. The type of heart disease could not be determined after thorough clinical investigation.

*Clinical Diagnosis:* Myocarditis with Adams-Stokes' syndrome.

### *Autopsy*

A complete postmortem examination was performed. Pathological changes, other than in the heart, consisted of congestive changes in the lungs, liver, spleen and kidneys. There was a considerable degree of edema of the brain.

### *Macroscopic Findings in the Heart*

The heart weighed 385 gm. There was a high degree of dilatation of the right and left ventricles. The ventricular muscle meas-



ured 1.5 cm. on the left and 0.8 cm. on the right. There was a striking, irregularly diffuse, grayish white discoloration of the parietal endocardium of both ventricles, which was much more extensive on the left.

Scattered throughout the mural endocardium were elevated and more or less circumscribed plaques which in a number of places were confluent. There was one large confluent plaque on the septum below the right coronary aortic valve and a smaller plaque just below the septum fibrosum. There was a large plaque situated on the right septal endocardium just below the pulmonary orifice. The surface of the papillary muscles was peppered with smaller plaques and areas of diffuse endocardial thickening. The aorta showed no evidence of arteriosclerosis or syphilis and both coronaries were patent and smooth along their entire visible course.

### *Histological Examination*

The striking change in the endocardium was found in the inner connective tissue layer. This was widened, particularly in the region of the plaques, and the elastic element played the dominant rôle in this change. In the areas of more or less diffuse endocardial thickening these elastic fibers were long and laid down in an orderly fashion parallel to the surface. As the plaques were formed they became shorter and thicker and, although intermeshing with each other to form a dense mass, maintained a general orderly arrangement. A thin outer zone of this layer did not seem to take part in this process and a narrow red band of connective tissue could be seen clearly just above the elastic lamina in the Weigert-Van Gieson preparations. Blood channels were scattered throughout this abnormal inner connective tissue layer. The elastic lamina was widened and from the outer border there was an outgrowth of elastic fibers. The architecture of the outer two layers was completely lost so that below the elastic lamina there was an irregular outgrowth of connective tissue which enmeshed degenerated muscle fibers, and in many places the conduction system was involved in this process and was badly damaged. Lymphocytes, scattered or in groups, were found in this outer layer and infiltrated the Purkinje system in many sections.

These lymphocytes, scattered, but mainly in miliary to submiliary sized foci, were found throughout the myocardium. Some of these

foci were enmeshed in a network of young fibroblasts. In addition there were foci similar to the latter which contained two or three giant cells. These giant cells were not of the Langhans type but resembled the foreign body form. They were elliptical in shape with an eosinophilic cytoplasm and many basophilic nuclei scattered around the periphery. These giant cell foci were for the most part perivascular, particularly around the medium sized venules. In some sections strands of connective tissue from the perivascular area were seen infiltrating into these foci.

In addition to a fatty degeneration of muscle fibers directly beneath the endocardium, degenerated fibers were occasionally found within the myocardium in nests of connective tissue and occasionally there were definite old myocardial scars. All of these scars were small. There was a general increase of the myocardial connective tissue, especially noticeable in the perivascular region. Only minimal arteriosclerotic changes were revealed by the fat stain.

Scattered foci of lymphocytes were present in the auricular myocardium but no giant cells or abnormal fibrosis were noted. The auricular endocardium was thickened, particularly on the left, with a definite increase in the elastic element. The aorta was normal in all of its layers.

### *Comment*

The damage found in the conduction system seems sufficient to explain the clinical evidence of heart block. The findings do not, however, explain the changing nature of the block. The factors responsible for such fluctuations are discussed in a forthcoming publication.<sup>8</sup>

It is such cases of endocardial sclerosis as this, occurring in relatively young individuals without evidence of arteriosclerosis or valvular disease, that have been infrequently described and studied. In this case the myocardium was the site of pronounced changes. These changes were characterized by an infiltration of lymphocytes which in foci, particularly perivenous, contained giant cells and young fibroblasts. There were no Aschoff bodies or other evidence of a rheumatic infection. In view of the giant cell foci tuberculosis or syphilis appears to be the outstanding possibility. The lack of the epithelioid element, the type of giant cell, the absence of tuberculosis elsewhere in the body, in addition to the fact that these foci in no

way resembled the architecture of the tubercle with its caseous center, definitely eliminate tuberculosis in its classical form. Against syphilis is the absence of a syphilitic mesaortitis, the non-gummatous character of the foci, and the negative Wassermann reaction. Consequently an isolated syphilitic myocarditis seems very unlikely.

Certainly it is reasonable to consider the various histopathological findings as different stages of the same process with fibrosis, particularly perivascular, as the end result. The picture as a whole definitely suggests a chronic infectious process, the nature of which cannot be determined from either the clinical or the pathological data.

In view of the findings the endocardial fibrosis must be considered secondary to the myocardial process. The fact that the endocardial change is mainly in the inner connective tissue layer, that the elastic fibers are more or less laid down orderly and, apparently, over a normal band of connective tissue, and also that the elastic lamina is unbroken, favor the conclusion that the fibrosis is a change compensatory for a subendocardial weakness. The changes in the lower layers are considered for the most part to be due to the myocardial process with pressure from the overlying fibrosis playing a possible rôle.

*CASE 2. Clinical Summary:* The patient was a 47 year old female who had always been well except for "double pneumonia" following a middle ear infection in 1926. Her final illness dated from 1933 and covered a period of 3 years, during which time she was more or less under continual hospital observation. The patient's original complaints were chronic fatigue, nervousness, diarrhea and loss of 20 pounds in weight.

Her illness was characterized by a continual elevation of the basal metabolic rate which resisted all medical treatment and which usually ranged between +40 and +50, although records as high as +80 and +100 were obtained on several occasions. In addition there were a hypotension averaging 90/50 mm. Hg., a fading brownish pigmentation of the skin, an inconstant prominence of the eyeballs, persistent diarrhea (3 to 5 movements daily), no gain in weight, non-valvular cardiac enlargement, and slowly progressive cardiac failure with attacks of pulmonary edema. There were no signs of infection. The urinary and blood findings, including the Wassermann reaction, showed no abnormality, excepting a moderate hypochromic anemia. The electrocardiogram tracings showed evidence of myocarditis and were suggestive of coronary infarction.

*Clinical Diagnoses:* Hyperthyroidism, myocardial failure and adrenal insufficiency.

### *Autopsy*

A complete postmortem examination was performed. The findings, exclusive of the heart, were colloid goiter without enlargement

or toxic changes, and congestion of the lungs, liver, spleen and kidneys. The adrenals and hypophysis showed no unusual histological changes. Foci of lymphocytes with foreign body giant cells were found in the lung alveoli and in the periportal connective tissue of the liver.

### *Macroscopic Findings in the Heart*

The heart weighed 420 gm. There was a high grade chronic dilatation of the left ventricle which was aneurysmal at the apex. There was an additional dilatation of the left auricle and a dilatation and hypertrophy of the right ventricle. The ventricular muscle measured 1.6 cm. on the left, and 0.9 cm. on the right. The valves were normal with the exception of the mitral. Along the free border of both leaflets of this valve pin-head sized fibrous nodules were found and the border of the valve was slightly thickened generally. The mitral orifice measured 10 cm.

The left parietal ventricular endocardium showed a striking, diffuse grayish white discoloration with plaque formation. The larger plaques were situated mainly in the posterior portion of the septum beneath the anterior leaflet of the mitral valve. There was a small plaque on the septum below the right coronary aortic valve and smaller scattered plaques were sprinkled over the papillary muscles. The right ventricular endocardium was minimally affected. There were a few small areas of grayish white thickening on the mural endocardium just below the attachment of the auricular leaflet of the tricuspid valve and on the papillary muscles below the pulmonic orifice. The left auricular endocardium seemed somewhat thickened. The aorta and coronary vessels showed no gross evidence of arteriosclerosis.

### *Histological Examination*

The myocardium of the left ventricle was riddled with small fibrous scars, a few of which were definitely perivascular. There was also an infrequent scattered collection of lymphocytes. Most of the blood vessels were distended and filled with blood, and fresh extravasated blood was seen in the connective tissue and between the muscle fibers. The right ventricular myocardium showed infrequent groups of three or four small scars. No Aschoff bodies were noted nor was arteriosclerosis of the smaller vessels found.

As in the previous case the inner connective tissue layer of the endocardium showed striking changes. The histopathological findings in this layer were comparable with those found in Case 1 except that the outer red band of connective tissue was not seen in the Weigert-Van Gieson preparations. The elastic lamina was widened but unbroken. The smooth muscle layer was not recognized as such so that under the elastic lamina there existed a layer of increased connective tissue which fused, for the most part, with scarred areas under the endocardium or invaded patches of muscle fibers, some of which were degenerating.

The auricular myocardium showed no abnormal changes while the auricular endocardium showed slight thickening. The nodules on the mitral valve were composed of scar tissue with a slight elastic element, but no Aschoff bodies were found. The aorta was normal.

### *Comment*

The pathological changes in this case are sufficiently clear to explain the cardiovascular aspects of the clinical picture. A thorough pathological examination failed, however, to reveal a basis for the metabolic features of the patient's illness. No explanation can be offered for the giant cell foci found in the lungs and liver. The fact that the patient in the past had worked as a silk weaver might, on the basis of inhalation of silk particles, have some bearing on their formation.

In regard to the myocardium it is unlikely that the scarring was due to arteriosclerosis. The small size of the scars, their dissemination through both the left and the right ventricular myocardium, and the absence of arteriosclerotic changes macroscopically and microscopically, leave only extremely remote possibilities based on an arteriosclerotic genesis. No Aschoff bodies were found and, in view of Grant's study,<sup>9</sup> there is little reason to assume that the changes in the mitral valve were rheumatic in origin. The myocardial scarring, however, could in no way be considered as a residuum of a rheumatic infection. The hemorrhage is regarded as a postmortem change.

The scarring was definitely an old process. Its small disseminated character and the history of a middle ear infection complicated by pneumonia strongly suggest the possibility that the histological picture is the end result of an embolic myocarditis. As for the lympho-

cytic infiltration, it is impossible to say whether it is a residuum of an infection or the result of an excessive effort of a badly damaged myocardium. Again, as in Case 1, no positive conclusions can be drawn but from the available evidence an infectious etiology for the myocardial lesion is regarded as the most probable.

The endocardial changes in this case closely resembled those found in the previous case. The main change was in the inner connective tissue layer of the endocardium and consisted of the laying down of elastic fibers in a more or less orderly fashion parallel to the surface. The elastic lamina was intact and definitely reinforced by new fibers. No changes were noted beneath the elastic lamina that could not be attributed to the myocardial process or to pressure. The possibility of the diffuse endocardial fibrosis resulting in any way from the process on the mitral leaflets is remote. The evidence of prolonged severe myocardial damage and the character of the histological changes in the endocardium again suggest a compensatory reaction on the part of the endocardium.

### DISCUSSION

In reviewing these 2 cases it can be said from the pathological evidence, as interpreted above, that in a group of cases of parietal endocardial sclerosis the changes may be considered as compensatory for a prolonged and severe subendocardial weakness. Although it so happened that in the cases reported here the myocardial process was most likely infectious in origin, it is felt that the same endocardial changes can occur from any process resulting in prolonged and severe myocardial debility. In these cases the change may be considered functional, but functional only in the well recognized physiological sense that every damaged organ protects itself as efficaciously as possible in order to survive. Thus, the functional change is not primary but secondary to an organic dysfunction of the myocardium, whatever its nature may be and whether it is histologically recognizable or not. In the present state of our knowledge it would only be purely hypothetical to discuss the possible factors that stimulate this protective response of the endocardium.

The plaque formation is interesting and always a striking feature of the macroscopic picture when it exists. The cases reported here do not allow a discussion of the inflammatory etiology of these plaques. That such may be the case, particularly in more or less

isolated plaques, seems quite probable. In the 2 cases presented here the plaque formation must be considered part of the general process, developing without inflammation of the endocardium. Consequently it is felt that the formation of fibrous plaques cannot be considered as a differential feature between functional and inflammatory endocardial sclerosis, as Böger suggests. No clue was obtained in this study as to why this compensatory response manifests itself in some cases by plaque formation. It is possible that this occurs over a particularly severely damaged subendocardial area.

### SUMMARY

1. Two cases of diffuse parietal endocardial sclerosis with plaque formation occurring in middle-aged individuals without arteriosclerosis are presented. In 1 case there was no evidence of valvular disease; in the other there was a slight degree of old rheumatic endocarditis of the mitral valve.

2. In each case evidence of prolonged and severe myocardial damage was present. In Case 1 the histopathological findings were unusual, being characterized by diffuse and focal lymphocytic infiltrations a few of which, particularly the perivenous, contained giant cells of the foreign body type. Fibrosis, especially the perivascular type, appeared to be the end stage of the process. In Case 2 diffusely disseminated small scars were found in the myocardium of both ventricles, particularly extensive in the left. Both cases are regarded as infectious in origin; the former, a chronic infection of unknown nature; the latter, the end stage of a previous metastatic myocarditis.

3. From the pathological findings it is concluded that in a group of cases of diffuse parietal endocardial sclerosis with plaque formation there is a compensatory protective change in the endocardium secondary to an organic subendocardial weakness. The organic nature of this weakness is not considered specific but may result from any process directly or indirectly affecting the myocardium which causes prolonged and severe weakness of the ventricular musculature.

4. It is concluded that parietal fibrous plaques may develop on such a basis and consequently cannot be used to differentiate between infectious and functional endocardial sclerosis.

NOTE: I wish to express my appreciation for the invaluable help given me in this study by Professor Aschoff. I also wish to thank Dr. Leo Müller of the Stadt Krankenhaus, Baden-Baden, for the opportunity of studying the heart in Case 1.

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## DESCRIPTION OF PLATES

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### PLATE 49

FIG. 1. Case 1. Left ventricle.

FIG. 2. Case 1. Right ventricle.



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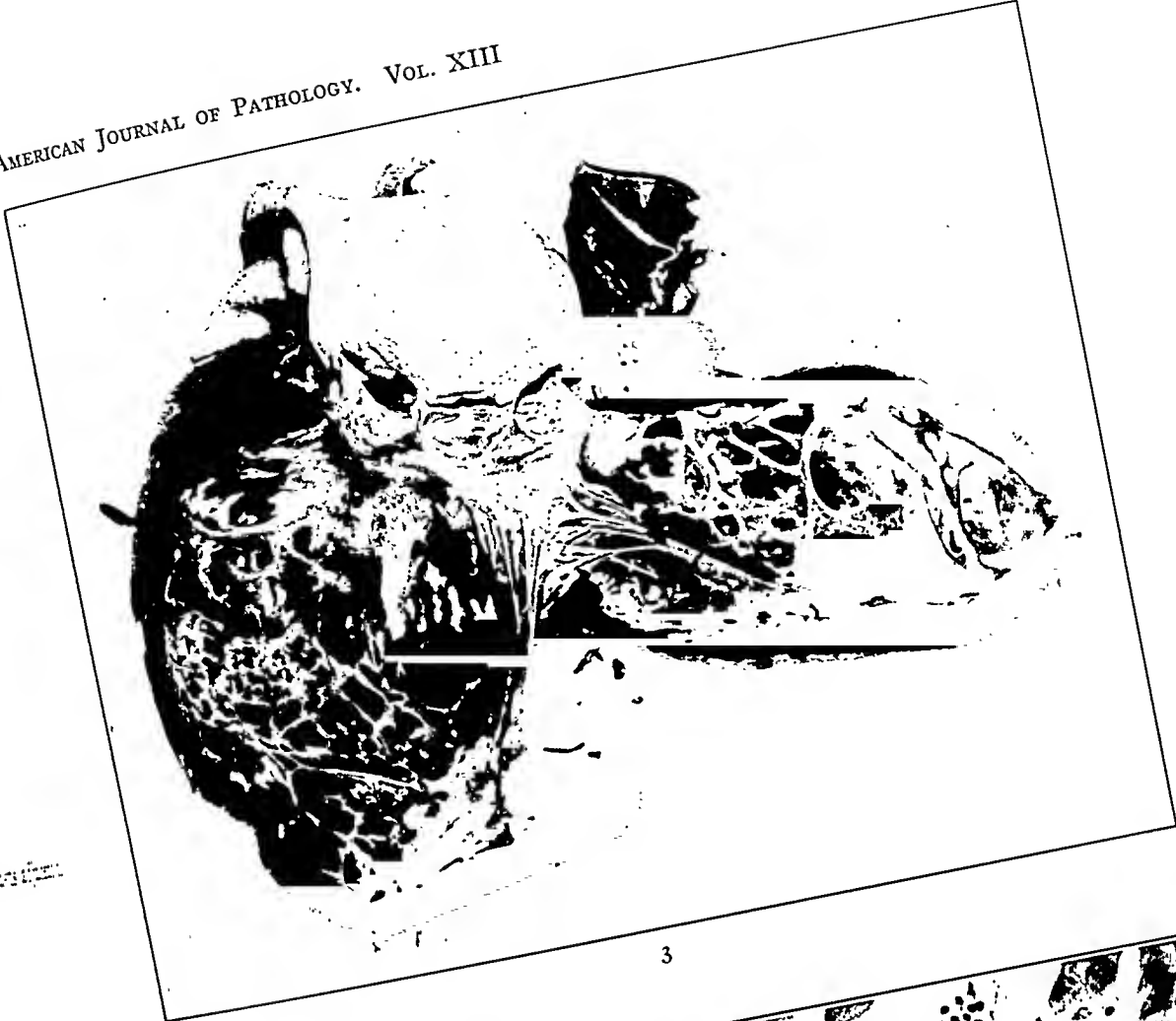
Diffuse Parietal Endocardial Sclerosis

Comeau

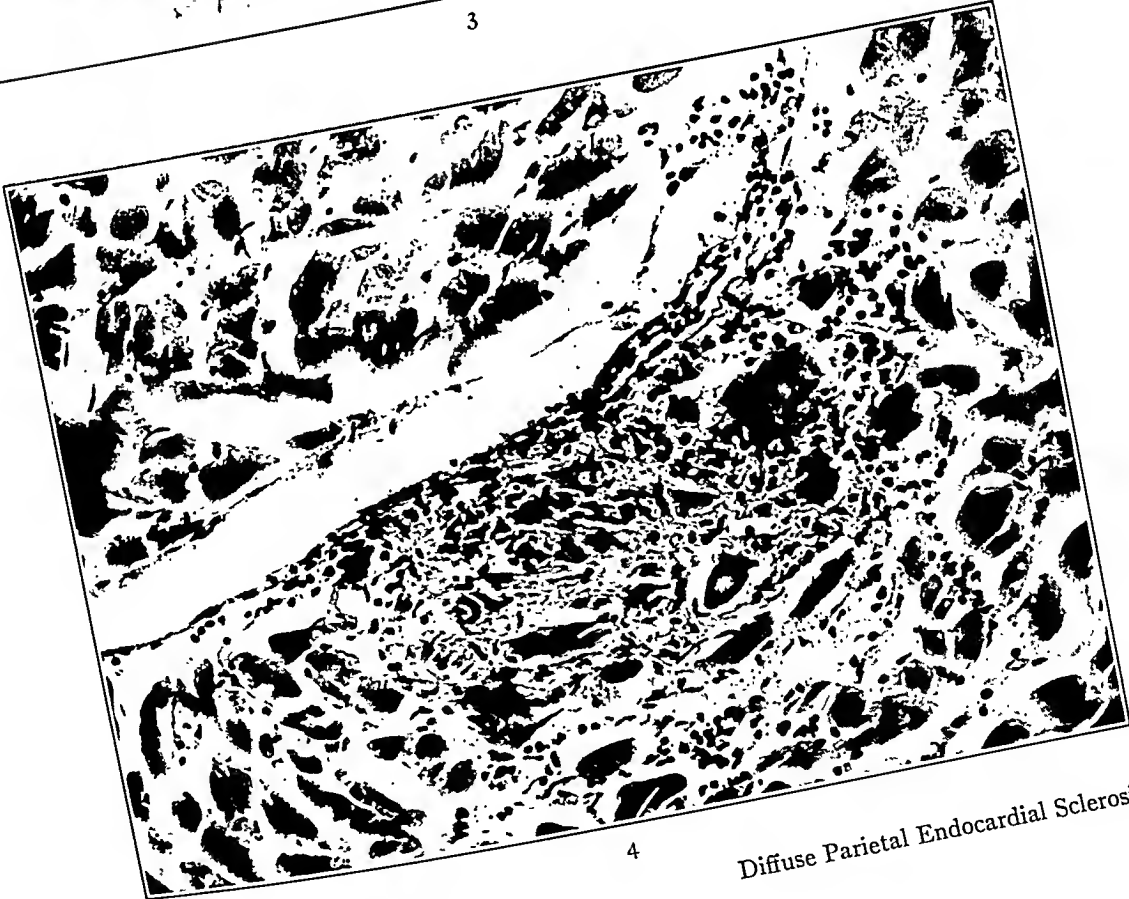
PLATE 50

FIG. 3. Case 2. Left ventricle.

FIG. 4. Case 1. Focus of lymphocytes, giant cells and fibroblasts adjacent to a venule. Hematoxylin-eosin stain.



3



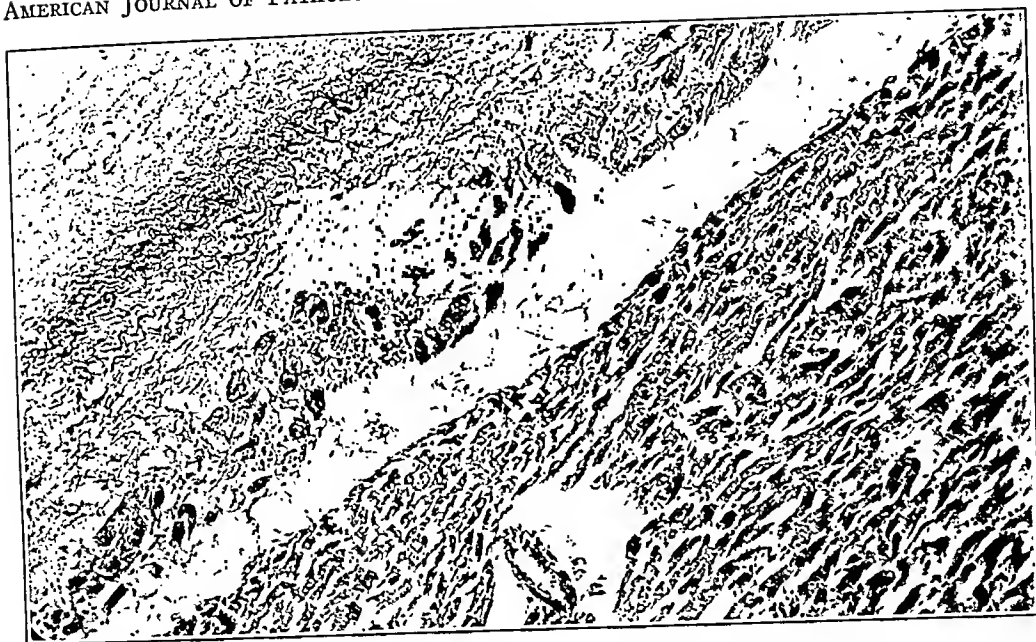
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Diffuse Parietal Endocardial Sclerosis

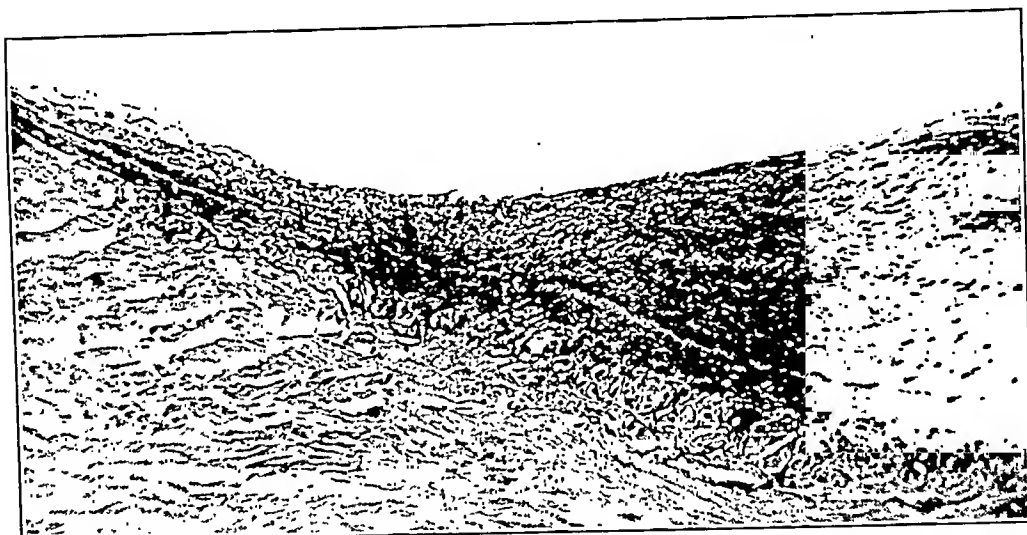
Comeau

## PLATE 51

- FIG. 5. Case 1. Showing a portion of the left branch of the conduction system invaded by fibroblasts and lymphocytes. Hematoxylin-eosin stain.
- FIG. 6. Case 1. Endocardium. The base of connective tissue above the elastic lamina can be plainly seen. Degenerated muscle fibers enmeshed in connective tissue are seen below the elastic lamina. Weigert's elastic tissue and Van Gieson's connective tissue stain.
- FIG. 7. Case 2. Endocardium. The parallel arrangement of the elastic fibers can be seen on the right. On the left they form a denser meshwork. Weigert's elastic tissue and Van Gieson's connective tissue stain.



5



6



7

Comeau

Diffuse Parietal Endocardial Sclerosis



# COMBINED INFANTILE AND ADULT COARCTATION OF AORTA WITH COINCIDENT OCCLUSION OF VENA CAVA SUPERIOR \*

## REPORT OF A CASE

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Coarctation of the aorta is not altogether a rare lesion. Dr. Maude Abbott<sup>1</sup> reported and reviewed 200 collected cases with autopsy in 1928. Mention was made in this article of 5 additional unpublished cases with autopsy. The relative infrequency of the condition may in part be accounted for by the failure of observers to record their cases. We have seen several prior to the case reported here and have reported none of them.

Two types of coarctation of the aorta are recognized: (1) the adult form in which the stenosis occurs at the level of the insertion of the ductus arteriosus or slightly proximal to this point; and (2) the infantile variety consisting merely of a narrowing of the descending portion of the aortic arch at the level of the inferior border of the origin of the left subclavian artery. As a rule one or the other type alone is present.

The etiological factors responsible for coarctation and the complications resulting from it have been adequately discussed by Abbott<sup>1</sup> and need not be mentioned here. It will be sufficient to mention at this time only that the most common findings associated with aortic coarctation are bicuspid aortic valve, with or without a superimposed bacterial endocarditis, subaortic stenosis, dilatation and hypertrophy of the heart, dilatation of the ascending aorta, arterial collateral circulation, anomalous origin of the arteries of the arch, hypoplasia of the aorta, persistent left superior vena cava, defects of the aortic septum, obliterative pericarditis and pleuritis, dissecting aneurysm of the aorta and aneurysmal dilatation of the cerebral vessels.

The above group of complications is usually associated with the adult form. Accompanying the infantile variety may be found more

\* Received for publication July 17, 1936.



TABLE I  
Analysis of 75 Cases of Coarctation of Aorta  
(1928-1935)

Author and year	Age and sex	Infantile type	Adult type	Collateral circulation	Associated anomalies	Secondary findings and other pathology	Cause of death	Clinical data
Green, F. H. K. <sup>3</sup> 1928	Male 21 yrs.	Mod. stenosis at isthmus admitting lead pencil, just distal lt. subclavian art.* D. A. oblit.		No mention	Bicuspid aortic valve	Mitotic congenital cerebral aneurysms	Sudden death; rupture congenital aneurysm basilar cerebral artery	
Jordan, F. L. J., and Ter Harr, F. I. <sup>3</sup> 1928	Not given		Atresia from origin lt. subclavian art. to oblit. D. A.	Increased	No mention	Hypertrophy & dilatation heart; fusiform aneurysm aortic arch above coarctation	Cardiac death	Pulsating carotids; capillary pulse; aortic insufficiency; Wassermann negative; loud interscapular murmur
Anderson, R. G. <sup>4</sup> 1928	Male 44 yrs.	Mod. stenosis aortic isthmus lumen 1.27 cm. diam. D. A. oblit.		Increased	Malignant endocarditis		Cardiac death; malignant endocarditis	
Rüster, H. <sup>8</sup> 1928	Not given	Stenosis aortic isthmus. D. A. oblit.		No mention	No mention			
Poynton, F. J., and Sheldon, W. P. H. <sup>4</sup> 1928	Male 8 yrs.	Mod. stenosis 1.1 cm. below lt. subclavian art. lumen narrowed to half original diam. D. A. oblit.		No mention	Bicuspid aortic valve; ulcerative aortitis beyond coarctation	Kidneys & spleen enlarged & contain recent infarcts	Positive hemolytic streptococcus blood culture	
Houck, G. H. <sup>7</sup> 1929 Case 1	Male 49 yrs.		Coarctation				Pneumonia	
Case 2	Male 29 yrs.		Coarctation				Miliary tuberculosis	
Weber, F. P., <sup>8</sup> and Knop, F. <sup>8</sup> 1929	Male 54 yrs.		Extreme stenosis aorta 1.3 cm. distal origin lt. subclavian art. at site insertion oblit. D. A.	Increased	Bicuspid aortic valve	Hypertrophy & dilatation heart; chronic oblit. pericarditis; chronic oblit. pleurisy; calcareous & verrucose cusps; calcification aortic ring	Lobar pneumonia	Ice block carrier
Bode, O. B., and Knop, F. <sup>9</sup> 1929 Case 1	Male 54 yrs.		Coarctation at oblit. D. A.	Increased			Cardiac death	
Case 2	Male 40 yrs.		Coarctation at oblit. D. A.	No mention		Hypertrophy left ventricle mainly	Cardiac death	Laborer — heavy work
Andresen, R. <sup>10</sup> 1929 Case 1	Male 47 yrs.	Stenosis aorta site oblit. D. A. lumen 3 mm. diam.		Increased	Dissecting aneurysm base ascending aorta	Hypertrophy & dilatation heart; coronary arteriosclerosis; slight dilatation upper portion descending aorta; meso-aortitis luica	Hemopericardium, rupture walls aneurysm	

Case 2	Male 18 yrs.		Mod. stenosis aorta after origin lt. sub- clavian & just below oblit. D. A. lumen size lead pencil	Moderate			Hypertrophy & dilatation heart	Drowning	
Case 3	Male 47 yrs.	Shelf-like stenosis directly beyond origin lt. sub- clavian art.	Extreme stenosis site oblit. D. A. lumen admits fine probe	Moderate			Fusiform aneurysmal dilatation descending aorta just beyond 2nd stenosis	Myocardial degen- eration	
Case 4	Male 48 yrs.		Mod. stenosis just be- yond site oblit. D. A. & 1.5 cm. beyond single vessel arising from arch	Increased		Bicuspid aortic valve; anomalous single vessel leaves aortic arch	Hypertrophy & dilatation heart; saccular aneurysmal dilatation ascending aorta; atheroma	Hanging	
Thomson, A. P., and Lamb, F. W. M. <sup>11</sup> 1929	Female 5 yrs.		Extreme stenosis 1" below origin lt. sub- clavian art. at site oblit. D. A.	No mention			Hypertrophy & dilatation heart — mostly on lt. side; dilata- tion ascending aorta; con- genital abnormality lt. kidney; hypoplasia rt. kidney	Cardiac death	
Sala, A. M., and Nacbarrie, I. <sup>12</sup> 1929	Male 6 yrs.		Extreme stenosis site insertion oblit. D. A.	None			Hypertrophy & dilatation lt. ventricle heart	Bilateral confluent bronchopneumonia; localized necrosis of lt. bronchus	
Fray, W. W. <sup>13</sup> 1930	Male 57 yrs.		Complete atresia site oblit. D. A.	Increased		Subaortic endocar- ditis with stenosis; ulcerative aortic endocarditis with extension into tricuspid ring	Hypertrophy & dilatation heart, mostly lt. ventricle; hypo- plasia abdominal aorta; sl. dilatation ascending aorta	Pulmonary throm- bosis; ulcerative aortic endocarditis	Good health until P. I. Onset with fever, dyspnea, weakness. Pulsat- ing neck veins in thoracic axillary & scapular artery. Post. bl. cult. <i>Str.</i> <i>viridans</i> . X-ray shows absent aortic knob & rib erosion
Terbruggen, A. <sup>14</sup> 1931	Not given		Atresia aorta site in- sertion oblit. D. A.	Moderate			Hypertrophy heart	Cardiac death	
Ernstene, A. C. <sup>15</sup> and Robins, S. A. <sup>15</sup> 1931	Male 47 yrs.		Atresia short distance below insertion oblit. D. A., stenosis 1.5 cm. on each side	Increased			Rib erosion limited to post. half ribs & inferior borders	Cerebral hemorrhage	Rib erosion shown by X-ray; cramps, weakness leg 5 yrs. Dyspnea, palpi- tation, pulsating neck vessels; B. P. upper extremity 164/90, lower ex- tremity 88/76; pulse tracings showed lag of fem- oral over radial

\* D. A. = ductus arteriosus

TABLE I—continued

Author and year	Age and sex	Infantile type	Adult type	Collateral circulation	Associated anomalies	Secondary findings and other pathology	Cause of death	Clinical data
Costa, A. <sup>16</sup> 1931	Female 54 yrs.		Atresia aorta site insertion oblit. D. A.	Increased		Aneurysmal dilatation 1st portion ascending aorta; hypertrophy, dilatation heart, mostly lt. side; 1 cm. from origin innominate art. showed aneurysma dilatation 8 cm. cir. with thrombotic deposits; 2 cm. caudal to atresia saccular aneurysmal dilatation post. rt. wall aorta; softened white rt. cerebral hemisphere	Cerebral embolism	Domestic servant
Ulrich, H. L. <sup>17</sup> 1932	Male 23 yrs.		Stenosis aorta site insertion patent D. A.	Increased		Hypoplasia descending aorta beyond coarctation, dilatation proximally; hypertrophy, dilatation heart; slight coronary arteriosclerosis; splenomegaly; portal cirrhosis; chr. pass. cong. viscera; ascites; fatty degen. liver	Cardiac death	
Lemon, W. S. <sup>18</sup> 1931	Male 22 yrs.		Stenosis where large patent D. A. entered aorta	No mention	Bicuspid aortic valve	Hypertrophy, dilatation heart; arteriosclerosis aorta & coronary artery; neurofibroma mediastinum		Pulsating vessels neck & thorax
Hein, G. E. <sup>19</sup> 1931 Case 1	Male 11 days	Atresia aorta proximal to widely patent D. A.		No mention	Defect 0.5 cm. diam. in interventricular septum	Dilatation, hypertrophy rt. heart; dilatation ascending aorta; foramen ovale anatomically patent		
Case 2	Male 32 yrs.	Narrowing aorta at isthmus				Autopsy showed syphilis		
Case 3	Male 60 yrs.	Narrowing aorta at isthmus			Aortic stenosis, calcification	Hypertrophy & dilatation heart; arteriosclerosis		
Abbott, M. E. <sup>20</sup> 1932	Female 34 yrs.	Narrowing aorta at isthmus	Extreme stenosis level insertion oblit. D. A. lumen 1.8 cm. cir.	Moderate	Bicuspid aortic valve (probably congen.) with calcified raphe; productive sclerosing aortic endocarditis	Hypertrophy & dilatation heart; dilatation ascending aorta; tricuspid & mitral stenosis		
Laubry, Ch., Routier, D., and Van Boogaert, A. <sup>21</sup> 1932	Male 42 yrs.	Stenosis aortic isthmus just below origin lt. subclavian art.		No mention			Pulmonary gangrene	B. P. 200/130 both arms; unable to record legs; pulsation neck vessels; previous pneumonia
Narr, F. C. and Wells, A. H. <sup>22</sup> 1933	Male 29 yrs.	Stenosis level of origin lt. subclavian art. D. A. oblit. at pulmonary end		No mention	Dissecting aneurysm ascending aorta	Hypertrophy, dilatation heart; arteriosclerosis coronary artery; fenestrated aortic valve	Hemopericardium. Rupture of wall of dissecting aneurysm 1 cm. from the base of aorta	

East, T. <sup>22</sup> 1932	Female 40 yrs.		Stenosis just below entrance oblit. D. A.	Increased	Rt. subclavian takes origin from lt. side aortic arch	Sl. hypertrophy lt. ventricle; sl. arteriosclerosis; infantile bicornate uterus; osteitis de- formans rt. tibia & skull	Fracture of skull	
Strong, G. F. <sup>24</sup> 1932 Case 1	Male 19 yrs.		Atresia at & below in- sertion oblit. D. A.	Moderate	Bicuspid aortic valve due to congenital fu- sion coronary cusps; rheumatic valvular endocarditis, sub- aortic stenosis; anomalous vessels	Hypertrophy & dilatation heart; dilatation descending aorta below constriction; hypoplasia descending aorta	Hemopericardium. Rupture of a di- lated and thinned ascending aorta	
Case 2	Male 39 yrs.		Stenosis 2 cm. inferior to origin lt. sub- clavian at site of in- sertion oblit. D. A.	Increased	Slight fusion coronary cusps	Enlarged heart; arteriosclerosis ascending aorta; anasarca; infarcts rt. lung	Cardiac death	Rib erosion shown by X-ray
Case 3	Female 12 yrs.		Stenosis level of in- sertion D. A., oblit. pulmonic side	None		Sl. hypertrophy & dilatation heart	Chronic nephritis, bilateral hydro- nephrosis, terminal bronchopneumonia	
Read, W. T. Jr., and Krum- haar, E. B. <sup>25</sup> 1932	Male 35 yrs.		Stenosis just below oblit. D. A. lumen 1.4 cm. diam.	Increased	Bicuspid aortic valve; anterior leaflet site of calcareous dep.	Hypertrophy, dilatation heart; hypoplasia aorta; coronary arteriosclerosis with myo- fibrosis; arteriolonephropathy	Cardiac death	B. P. 200 mm. Hg.; cyanotic & uncon- scious; fluid pour- ing from mouth
Carnett, J. B., and Howell, J. C. <sup>26</sup> 1932	Male 75 yrs.		Stenosis aorta site oblit. D. A.	Increased		Dilatation ascending aorta; hypoplasia aorta distal to coarctation; mod. hyper- trophy & dilatation aorta	Terminal broncho- pneumonia, pul- monary edema	Wass. Neg.; dysp- nea. B. P. rt. arm 180/80; lt. arm 160/80; rt. leg 140/96; lt. leg 100/30; X-rays show notching of ribs
Schleickat, O. <sup>27</sup> 1933	Male 44 yrs.	Mod. stenosis aortic isthmus				Hypertrophy & dilatation heart	Terminal broncho- pneumonia	Wassermann positive
Root, J. H. <sup>28</sup> 1933	Male 13 days	Stenosis aorta be- tween openings left carotid & lt. subclavian art. lumen 8 mm. cir. D. A. not identified		No mention	Bicuspid aortic valve, fusion coronary cusps	Hypertrophy & dilatation heart	Cardiac death	
Pozzan, A. <sup>29</sup> 1933	Male 42 yrs.		Atresia aorta site oblit. D. A.	Increased	Bicuspid aortic valve showing thick cal- careous plate involving portion pars membranacea septi; dissecting aneurysm ascend- ing aorta	Hypertrophy & dilatation heart mostly lt. side	Hemopericardium, rupture dissecting aneurysm aorta at base	Robust, athletic type

TABLE I—continued

Author and year	Age and sex	Infantile type	Adult type	Collateral circulation	Associated anomalies	Secondary findings and other pathology	Cause of death	Clinical data
Lewis, T. <sup>30</sup> 1933 Case 1	Male 43 yrs.		Stenosis aorta immediately beyond insertion oblit. D. A. Lumen a slit 0.5 cm. X 1.5 cm.	Increased	Patches sessile vegetations aortic cusps (organized subacute bacterial endocarditis) commissures fused	Hypertrophy, dilatation heart, mostly lt. side; dilatation ascending aorta; bilateral fibinous pleurisy, numerous infarcts both lungs; anasarca	Cardiac death	
Case 2	Male 49 yrs.	Aorta narrows beyond left subclavian art.	Atresia immediately beyond insertion oblit. D. A.	Increased	Annular sclerosis aortic cusps	Sl. dilatation ascending aorta; hypertrophy, dilatation heart, mainly left ventricle; coronary arteriosclerosis	Cardiac death	Systolic murmur over chief vessels; no pulsations leg artery; large pulsating vessels neck, interscapular & axillary regions
Case 3	Male 68 yrs.		Stenosis aorta 2 cm. beyond origin lt. subclavian art. D. A. oblit. Lumen aorta at stenosis 6 X 11 mm.	Moderate	Bicuspid aortic valve, fusion coronary cusps	Hypertrophy & dilatation heart, mainly left ventricle; arteriosclerosis; frontal convulsions brain atrophied; patchy myofibrosis heart; chr. pass. cong. lungs; stones gall bladder	Coronary arteriosclerosis, thrombosis	
Giroux, M. and Jobin, J. B. <sup>11</sup> 1933	Female 20 yrs.		Atresia aorta at level oblit. D. A.	No mention	Foramen ovale patent	Hypertrophy & dilatation heart; vegetative mitral endocarditis (subacute bacterial); hypoplasia abdominal aorta; interstitial degenerative myocarditis; ascites; chr. pass. cong. viscera; small thrombus right axillary artery	Cardiac death	B. P. 220/40; dyspnea; peripheral edema
Stewart, H. L., and Ballet, S. <sup>31</sup> 1934	Male 26 yrs		Stenosis immediately below insertion oblit. D. A. Lumen admits bristle	Increased	Dissecting aneurysm ascending aorta	Hypoplasia descending aorta; no syphilis or arteriosclerosis	Hemopericardium. Ruptured wall dissecting aneurysm at base aorta	
Evans, W. <sup>32</sup> 1933 Case 1	Male 2 wks.	Stenosis aortic arch proximal to origin lt. common carotid. D. A. widely patent, 0.15 cm.		No mention	Left vertebral artery arises from narrowed portion aortic arch; mitral orifice absent, no communication between lt. ventricle and auricle; patent interventricular septum (0.5 cm.), foramen ovale patent (0.5 cm.)	Rt. heart hypertrophy & dilatation; Meckel's diverticulum	Cardiac death	
Case 2	Male 30 yrs.	Consid. stenosis aorta between orifices lt. common carotid & lt. subclavian art. D. A. oblit. (0.8 cm. cir.)		No mention		Hypertrophy & dilatation heart; anasarca; cyanosis; gen. arteriosclerosis	Cardiac death	

Case 3	Female 2 wks.	Stenosis aortic isthmus immediately beyond orifice lt. subclavian art. D. A. patent (0.8 cm. cir.)		No mention	Foramen ovale patent	Hypertrophy & dilatation heart	Bronchopneumonia, purulent bronchitis, extensive areas collapse
Case 4	Female 2 days	Stenosis aortic arch & isthmus. D. A. widely patent		No mention	Foramen ovale patent	Rt. ventricle hypertrophy, dilatation	Heart failure
Case 5	Male 8 yrs.		Stenosis aorta opposite center orifice of patent D. A. (0.5 cm. diam.)	No mention		Rt. heart hypertrophy, lt. heart hypoplasia; hypertrophy pulmonary artery; tuberculosis tracheobronchial lymph nodes; anasarca; cyanosis	Cardiac death
Case 6	Male 5 days	Stenosis at isthmus just distal to origin lt. subclavian art. D. A. patent (0.15 cm. diam.)		No mention	Foramen ovale patent (5 by 4 mm.); rt. auricle receives both rt. and lt. vena cava; lt. sup. auricle by way coronary sinus passing behind pulmonary vein	Congenital abnormality jejunum	Intestinal obstruction
Case 7	Female 1 day	Stenosis proximal to opening widely dilated D. A. (1.5 mm. cir.) Lumen aorta 1.1 cm. cir.		No mention	Patent foramen ovale (0.2 cm.); lt. vertebral artery arising from arch aorta	Hypertrophy rt. ventricle, auricle	Cardiac death
Case 8	Female 5 wks.	Stenosis above orifice patent D. A. (0.7 cm. cir.)		No mention	Foramen ovale patent (0.1 cm.); patent interventricular septum (0.7 by 0.3 cm.)	Hypertrophy & dilatation, right heart	Cardiac death
Case 10	Male 3 mos.		Stenosis aorta immediately beyond insertion oblit. D. A.	No mention	Foramen ovale patent (0.1 cm.)	Slight dilatation & hypertrophy lt. ventricle; no rib erosion	Cardiac death; bronchopneumonia
Case 11	Male 16 yrs.		Stenosis aorta just below 1st pair bronchial arteries. D. A. oblit. Lumen admits fine probe	Moderate		Hypertrophy lt. ventricle; flecks atheroma in ascending aorta, none in descending	Bronchopneumonia
Case 12	Male 60 yrs.		Stenosis site insertion oblit. D. A.	Moderate	Lt. vertebral artery takes origin from aortic arch between lt. common carotid & lt. subclavian art.	Dilatation upper portion descending aorta; carcinoma pylorus; brown atrophy heart; atherosclerosis ascending aorta	Pulmonary embolism; thrombosis venae comites meningeal artery, thrombosis artery, lower limbs

TABLE I — continued

Author and year	Age and sex	Infantile type	Adult type	Collateral circulation	Associated anomalies	Secondary findings and other pathology	Cause of death	Clinical data
Case 13	Female 18 mos.		Stenosis aorta site insertion oblit. D. A.	No mention		Thrombosis posterior base superior longitudinal sinus & adjacent 2 cm. both lateral sinuses	Heart failure; bronchopneumonia; foreign body in bronchus	
Case 14	Male 35 yrs.		Stenosis site oblit. D. A. (admits fine probe)	Increased	Bicuspid aortic valve fusion rt. & lt. anterior cusps; calcification valves	Hypertrophy & dilatation lt. ventricle; acute fibrinous pericarditis; septic erosion small pyogenic saccular aneurysm aorta immediately above aortic commissure; vegetations margins of aneurysm; anasarca; septic splenec; anemic infarct kidneys	Hemopericardium; rupture mycotic aneurysm of aorta	
Case 15	Female 6 yrs.		Stenosis aortic arch site D. A. & isthmus, pulmonary portion D. A. closed. Aneurysm aortic portion D. A.	No mention		Sl. rt. heart hypertrophy & dilatation; chr. abscess middle lobe rt. lung with focal empyema; purulent infiltration wall D. A., wall false aneurysm & adjacent pulmonary art.; erosion upper lobe of lt. lung by aneurysm D. A.; false aneurysm wall aorta beneath pericardium & to left of roots great vessels lined by organized blood clot	Hemoptysis, bronchopneumonia; rupture mycotic aneurysm D. A. into lung	
Case 16	Male 23 yrs.		Stenosis aorta at level insertion oblit. D. A.	Increased	Bicuspid aortic valve, interventricular septum filled with vegetations; con-sid. destruction common rt. & post. cusps; vegetations spread over aortic cusps; anomalous vessel	Hypertrophy & dilatation of heart; vegetations filling smooth lined aneurysm (1.5 cm. diam.) in ant. part base aortic cusp mitral valve; anasarca, generalized sl. arteriosclerosis	Heart failure; aortic incompetence; sub-acute bacterial endocarditis	
Case 17	Male 35 yrs.		Stenosis 2.5 cm. beyond lt. subclavian art. (admits probe) D. A. oblit.	Increased		Saccular aneurysm anterior wall ascending aorta filled with thrombus & showing partial calcification of wall; dilatation descending thoracic aorta (5.0 cm. cir.); anasarca; sl. atherosclerosis distal to stenosis in aorta	Hemorrhage into pericardial adhesions; rupture aneurysmal wall ascending aorta	
Case 19	Male 32 yrs.		Atresia aortic arch. D. A. oblit.	Increased	Bicuspid aortic valve. Few recent vegetations slightly thickened aortic valve	Hypertrophy & dilatation of heart; dilatation aortic arch, ascending aorta & commissures; gen. arteriosclerosis	Heart failure; aortic incompetence; acute rheumatic endocarditis	

Taylor, E. F. <sup>34</sup> 1934	Female 59 yrs.		Atresia aorta level oblit. D. A.	Increased	Bicuspid aortic valve; calcified, fibrosed, fusion of posterolateral anterior cusp	Gen. arteriosclerosis; aneurysm 1st portion ascending aorta; hypertrophy & dilatation heart	Hemopericardium; rupture aneurysm ascending aorta	"Rheumatic pains," back & legs many years; dyspnea
Pierce, W. F. <sup>35</sup> 1934	Male 25 yrs.		Atresia aorta 18.2 cm. beyond cusps. D. A. oblit.	No mention		Empyema scars right pleural cavity	Gangrenous abscess mediastinum com- municating with thoracic portion aorta & esophagus	
Bargi, L. <sup>36</sup> 1934	Female 19 yrs.	Spur just below ori- gin lt. subclavian art. producing stenosis	Moderate stenosis aorta site D. A. (1.5 cm. cir.) D. A. oblit.	Increased	Bicuspid aortic valve; fusion of lt. post. & ant. cusps	Saccular aneurysmal dilatation between two levels of stenosis; hypertrophy & dilatation heart, mostly lt. side; stomach full of blood. Just caudal to 2nd stenotic area aorta an aneurysm size of a cherry contained a thrombus; lower margin shows rupture	Rupture thoracic aortic aneurysm into esophagus & hemorrhage	History rheumatic fever; vague pains legs & shoulders; many attacks fever; dispropor- tion between rad- ial & femoral pulse; murmur between scapulae
Brown, J. W. <sup>37</sup> 1934	Female 30 yrs.		Atresia aorta; beyond origin lt. subclavian & just distal oblit. D. A.	Increased	Bicuspid valve; rt. cusp shows mass yellow friable vegetations arising from root of aorta; projecting into right auricle small unruptured my- cotic aneurysm; anomalous vessels originating distal to coarctation	Hypertrophy & dilatation of heart, mostly lt. side. Dilata- tion ascending aorta; enlarged thyroid gland	Cardiac failure	Dyspnea & swelling legs; B. P. right arm 180/20, left 150/20; systolic B. P. leg 100; up- per extremities warm & well col- ored. Lower ex- tremities cold, bluish & edema- tous; notching of ribs; violent pul- sations neck vessels
Hardaway, R. M., and Sawyer, H. P. <sup>38</sup> 1934	Male 38 yrs.	Stenosis 2 cm. be- low origin lt. common carotid & lt. subclavian art. 1 cm. above oblit. D. A.		Increased	No innominate artery; rt. and lt. subclavian arteries originate from arch; anomalous origin of lt. sub- clavian artery near end descending arch	Hypertrophy & dilatation heart; rib erosion	Heart failure	B. P. 300/140 both arms
Rumold, M. J., and Schwartz, E. <sup>39</sup> 1934	Male 18 yrs.		Constriction aorta just distal insertion oblit. D. A., atresia	No mention	Bicuspid aortic valves; dissecting aneurysm ascend- ing aorta contain- ing blood clot	Hypertrophy & dilatation aorta; dilatation ascending aorta; patchy myofibrosis	Hemopericardium; rupture walls dis- secting aneurysm ascending aorta	Dilated vessels neck, chest & scapular regions
Narr, F. C., and Johnson, E. T. <sup>40</sup> 1934	Male 7 yrs.		5 cm. from base of aorta, vessel be- came narrow & stenosed. At level oblit. D. A. stenosis still present but mod.	No mention	Dissecting aneurysm ascending aorta	About 3 cm. above base of aorta was a rent partially filled with blood clot; at edges of opening 2 large vegetations; ulceration intima ascending aorta	Hemopericardium, rupture ascending aorta	



TABLE I—continued

Author and year	Age and sex	Infantile type	Adult type	Collateral circulation	Associated anomalies	Secondary findings and other pathology	Cause of death	Clinical data
Levine, H. D. <sup>41</sup> 1934	Male 10 mos.	Stenosis aorta between origin lt. subclavian & insertion oblit. D. A. (1.5 cm. cir.)		No mention		Hypertrophy & dilatation heart; atelectasis left lung; fibrosis endocardium left ventricle	Cardiac death	
Kellogg, F., and Biskind, G. R. <sup>42</sup> 1934	Male 16 yrs.		Stenosis 2 cm. below origin lt. subclavian art. & immediately above patent D. A. which admits bristle	Moderate	Anomalous bifid coronary artery behind rt. cusp; bicuspid aortic valve; valve partially destroyed by crumbling vegetations; rupture rt. cusp	Hypertrophy & dilatation lt. ventricle; mycotic aneurysm descending thoracic aorta & superior mesenteric artery	Subacute bacterial endocarditis; terminal bronchopneumonia; recent localized peritonitis	
Beatty, J. F. <sup>43</sup> 1934	Male 22 yrs.		Atresia aorta level oblit. D. A.	No mention		Hypertrophy & dilatation heart		Complete absence pulse in vessels lower limb & abdominal aorta
Ballantyne, E. N. <sup>44</sup> 1935 Case 1	Male 21½ hrs.	Immediately beyond origin lt. subclavian art. aorta narrowed, admits only point of dissecting needle. Stenosis above patent D. A.		No mention		Hypertrophy & dilatation heart	Cardiac death	
Case 2	Male 12 wks.	Atresia just beyond origin lt. subclavian art. D. A. patent (3 mm.)		No mention	Common right ventricle	Hypertrophy rt. & lt. auricles & common ventricle; dilatation ascending aorta	Cardiac death	
Case 3	Male 4 mos.	Stenosis just proximal to oblit. D. A.		No mention	Foramen ovale anatomically patent; also pars membranacea interventricular septum (8 mm.)	Hypertrophy & dilatation heart	Cardiac death	
Farris, H. A. <sup>45</sup> 1935	Male 11 yrs.		Stenosis site D. A. (admits probe) D. A. oblit.	Moderate	Bicuspid aortic valve; cusps thickened; contained organized scar tissue about base each valve—ventricular side	Erosion 7th & 8th ribs; hypertrophy & dilatation heart, mostly lt. side; dilatation ascending aorta; obliterative pleuritis—rt.; dilatation & thrombosis descending aorta just beyond atresia	Subarachnoid hemorrhage	X-rays showed absence aortic knob; erosion ribs; pulsating neck vessels
Jacobson, C. J. <sup>46</sup> 1935	Female 35 yrs.		Stenosis aorta just below insertion oblit. D. A.	Increased		Hypertrophy & dilatation heart; congestion liver & kidneys	Cardiac death; hemothorax; perforation diaphragm on concave side arch aorta	

Pozzan, A. <sup>47</sup> 1935	Female 69 yrs.	Atresia aorta 3 cm. beyond origin lt. subclavian art. aneurysmal niche in superior wall aorta at site oblit. D. A.	Increased	Foramen ovale patent	Hypertrophy & dilatation heart, mostly lt. side; aneurysmal niche in superior wall aorta at site D. A. contains thrombus. Edema legs; cyanosis; gen. arteriosclerosis. 1 cm. below orifices renal arteries aorta transformed into rigid cord oblit. by sclerotic connective tissue & calcium salts. Common iliac, ext. iliacs, hypogastrics on both sides thrombosed, as were the femoral arteries as far as popliteals	Cardiac death	Onset sudden pain in legs at night
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complex anomalies such as a biloculate or triloculate heart, patent foramen ovale, patent interventricular septum, transposition of the arterial trunks, pulmonary atresia and so on.

The case of coarctation we are reporting is unique, not only because of the presence of a double stricture of the aorta, one at the site for infantile coarctation and the second at the location for the adult form, but also on account of coexistent chronic occlusion of the superior vena cava, which necessitated as extensive a collateral venous circulation as was demanded of the arterial tree by the high grade aortic stenosis.

Of all the cases in the available literature since Abbott's summary<sup>1</sup> only 3 in addition to ours exhibit a double stricture at and proximal to the aortic termination of the obliterated ductus. In these cases the descending aorta first narrowed abruptly a slight distance beyond the origin of the left subclavian artery, only to become constricted again at the level of the closed ligamentum arteriosum by a thickened, smooth, internal stenotic ring or complete atresia.<sup>10, 20, 36</sup>

In Table I we have attempted to tabulate all cases of coarctation of the aorta appearing since Abbott's last summary in 1928.

### REPORT OF CASE

*Clinical History:* R. C., a 67 year old painter, entered the Multnomah County Hospital in November, 1935, complaining of dysuria, nocturia, frequency, incontinence and constant hematuria. At the age of 22 years he had had gonorrheal urethritis, and a year later a chancre treated by antiluetic therapy which was continued for 4 years. The patient had had lobar pneumonia twice and pleurisy once. When admitted there was no chest pain, palpitation or tachycardia, although he had noted dyspnea on moderate exertion and swelling of the feet and ankles.

*Physical Examination:* The blood pressure was 180/80 left arm, 180/85 right arm; the pulse 98, temperature 98.6° F., and the respirations 20. The patient was of the hypersthenic type, and lay quietly in bed, although grimacing and grunting every few minutes. The arms, chest and abdomen were covered with varicose veins. The right pupil measured 1.5 mm. in diameter, the left 3.0 mm.; both were round, regular, and did not react to light but showed a fair reaction to accommodation. The neck showed a bilateral, symmetrical, slight lymphadenopathy. The trachea was in the midline. The chest cage was symmetrical with flaring at the costal margins. The expansion was poor but equal. The breath tones were sharp and vesicular. No râles could be heard on ordinary breathing or expiratory cough. The heart presented an aortic configuration. The apex beat was in the sixth interspace 14 cm. from the midline and was localized by a slight thrust. There was slight impairment of resonance to percussion at the upper and lower sternal areas. A blowing systolic murmur was heard over all valve areas. The M<sub>2</sub> was weak, while A<sub>2</sub> had a loud, ringing metal-

lic sound. The rhythm was regular with an occasional extrasystole. The varicose veins of the arms, chest and abdomen all converged toward the midchest area. The abdomen was rotund, not rigid, and was free from masses or areas of tenderness. The liver was 3 cm. below the costal margin and its edges were smooth. The kidneys and spleen were not palpable. There was tenderness and pain, which radiated down the penis when pressure was made over the bladder area. The prostate was enlarged, irregular, soft and not tender. A firm, non-painful nodule 0.5 cm. in diameter was palpated posteriorly in the left lobe of the prostate. The reflexes were all very weak except for the biceps, triceps, periosteal and radial, which showed increased reactivity.

*Laboratory Examinations:* Urine: red, alkaline, slightly cloudy with a specific gravity of 1.017, albumin 3 plus, reduction negative, acetone negative, triple phosphates 1 plus, occasional pus cells, red blood cells 1 plus, bacterial 1 plus, and mucus 4 plus.

Blood: hemoglobin 81.8 per cent (Sahli), red blood corpuscles 3,910,000, white blood corpuscles 8500, polymorphonuclears 77 per cent, basophiles 1 per cent, small lymphocytes 5 per cent, mononuclears 4 per cent, staff 12 per cent, degenerated forms 1 per cent, sedimentation rate 45/118. Urea nitrogen 22.5 mg., and alkali reserve 71.8. Kolmer and Kahn tests negative.

*X-Ray:* The first roentgenogram of the chest, made with a portable machine, disclosed nothing beyond slight infiltration apparently along the interlobar fissure on the right side, suggesting the possibility of an interlobar effusion or pneumonic consolidation in the middle or posterior portion of the lower lobe, and definite notching of the inferior margins of the ribs posteriorly. Later chest films showed definite consolidation in the upper lobe of the right lung in addition to notching of the ribs.

*Course of Illness:* A retention catheter was installed and fluids were being forced orally in preparation for cystoscopy when the patient suddenly developed cyanosis of the nose, lips and ears, and a mild edema of the feet. The temperature was 102° F.; respirations 36; pulse 116, full and regular. There was no complaint of pain in the chest. Examination revealed a few, moist, basal râles posteriorly. Under rigorous antipneumonic therapy the temperature slowly fell to 99° F. During the next 3 days the cyanosis disappeared, the respirations decreased to between 12 and 18, and the blood urea nitrogen from 45 to 32 mg. Nevertheless, the patient seemed to be failing and on the 4th day the temperature began to rise, reaching 106° F. on the 6th day of hospitalization. Moist râles were heard all over the chest. Death occurred suddenly on this day.

The clinical diagnoses were: carcinoma of the urinary bladder with retention, cystitis and ascending pyelitis; arteriosclerotic heart disease with hypertrophy and dilatation of all the chambers, regular rhythm with extrasystoles Grade 2-A; neurosyphilis (tabetic type); and bronchopneumonia.

### POSTMORTEM EXAMINATION

The body is well nourished and of the muscular type. The neck appears edematous but otherwise is unchanged. The thorax is symmetrical and well developed. Over the chest and abdomen are numerous interlacing, distended superficial veins. The extremities are symmetrical, muscular and well developed.

There is no free fluid in the peritoneal cavity. The urinary bladder is distended with turbid urine. The mucosa is everywhere covered with a dull, gray-green shaggy pseudomembrane. On the posterior wall of the fundus is a papilloma measuring about 2.3 cm. in diameter and capped by a false membrane. The prostate is small and not obstructive. The internal hemorrhoidal plexus of veins shows slight dilatation. The abdominal aorta and the iliac arteries have a smaller than normal caliber.

The lungs are bound down everywhere by fibrous connective tissue adhesions and are freed with difficulty. In the dissection the internal mammary arteries are severed and are found to be abnormally prominent. Their lumens at the point of exit from the mediastinum measure approximately 0.75 cm. in diameter. The intima of both vessels displays considerable arteriosclerosis. The intercostal arteries lie in deep grooves along the inferior borders of the ribs. The grooving is most prominent posteriorly. Over the diaphragm and the base of the pericardium the fibrous adhesions are considerably thickened and contain areas of calcification. The pericardial sac is obliterated by fibrous adhesions and the pericardium is bound externally to all the surrounding structures. The entire lower lobe of the left lung is consolidated. The upper lobe is, for the most part, air-containing; inferiorly it shows focal areas of firmness. The right lung is air-containing, except at the base where a consolidation is noted. At the level of the tracheal bifurcation are three small traction diverticula, and 5 cm. superiorly are two more. The lower ones are attached to anthracotic and calcified nodes. About the posterior reflections of the diaphragm are seen areas of extreme fibrosis and some calcification. The right adrenal shows at the lateral boundary within the cortex a firm white to yellow area with a few small, white, chalk-like dots in its substance. The right kidney is considerably atrophied, cuts with increased resistance and presents a pitted granular and cystic cortical surface; aberrant arteries go to the lower pole. The left kidney is of moderate size. The cortical surface is smooth. The cut surface shows no distortion of cortical markings. The renal arteries show considerable arteriosclerosis.

The heart is enlarged on both the right and left sides and has a prominent pulmonary conus. It is everywhere of uniform firmness. The maximum transverse diameter is 17 cm. The thickness of the right ventricle at the apex is 0.5 cm., at the base 1.5 cm., and anteri-

only 1.5 cm. The circumference of the pulmonary ring is 8 cm. The trabeculae carneae of the right ventricle are enlarged and flattened. The anterior papillary muscle is hypertrophied. The right ventricle is considerably dilated. The left ventricle shows pronounced hypertrophy and dilatation. At the apex it measures 1.5 cm. in thickness and at the base 3 cm. The auricular appendages are of normal size and show no gross changes. The pulmonary arteries display well defined arteriosclerosis. The coronary arteries show considerable arteriosclerosis with lumens that are often slit-like but patent. The myocardium of the left ventricle on tangential section exhibits a diffuse patchy replacement fibrosis of the musculature. The endocardium of the left ventricle is slightly thickened and moderately opaque. There is seen a subaortic area of calcification with the formation of an annular concrescence of calcium deposits covered by endothelium. This is found at a distance of 0.5 cm. inferior to the right posterior cusp. Anteriorly it projects upward to involve the anterior cusp and extends into its sinus of Valsalva. The aortic valve is bicuspid. The coronary cusps are congenitally fused, for no nodule of Arantius, raphe or other septum-like structure divides the cusp into two portions. The anterior cusp measures 5 cm. along its free margin. The right posterior cusp measures 4 cm. along the free border. The coronary ostia are of moderate size and lie behind the larger and anteriorly situated cusp. The right coronary orifice lies to the extreme right and anterior half of the larger sinus of Valsalva, while the left is in the extreme posterior angle. The foramen ovale and ductus arteriosus are closed. The aorta is normally situated, bulges slightly to the right and forms a slight aneurysmal dilatation 4.5 cm. in diameter. At the beginning of the transverse arch the aorta becomes constricted and reduced to a diameter of 1 cm. at the site of the infantile isthmus, as compared to 1.8 cm. proximal to the ostium of the subclavian artery. Here the lumen is concentrically constricted by a symmetrical, smooth surfaced ridge or shelf (Figs. 1 and 2). The great size of the left subclavian artery makes it appear to be almost a continuation of the aorta for it too has a diameter of 1.8 cm. Beyond the first coarctation is a slight, but distinct bulbous sacculation of the aorta 2 cm. in diameter. At a point 2 cm. distal to the first coarctation and to the sacculum referred to, the descending aortic arch presents externally a shallow annular constriction which coincides internally with an almost complete occlusion of the lumen

by an endothelial covered diaphragm, at the center of which is a minute slit 0.15 cm. in length and barely wide enough to transmit light and water with difficulty. On the medial aspect at the level of the second coarctation the aorta is joined by the ligamentum arteriosum. The descending thoracic aorta just caudal to the second point of stenosis shows a slight diffuse saccular dilatation, the lumen measuring 3.5 cm. in diameter. At the level of the celiac artery the aortic lumen measures 1.8 cm. and at the bifurcation decreases to 1.5 cm. Within an area of 3 square cm., immediately caudal to the lower coarctation, the ostia of ten vessels are seen. These vessels are paired, located symmetrically on the posterior wall and apparently are intercostal arteries. The upper four pair are hypertrophied and dilated, having lumens ranging from 0.3 to 0.7 cm. in diameter. The internal mammary arteries are greatly enlarged and at a point where they leave the mediastinum and course along the chest plate the lumen is found to measure on the right 0.5 cm. and on the left 0.8 cm. The lumen of the left subclavian artery at a distance of 3 cm. from its origin has a diameter of 2 cm. The left common carotid at a similar distance measures 0.8 cm. in diameter. The lumen of the innominate artery is 2.2 cm. in diameter. All arteries leaving the heart and the aortic arch show an advanced degree of arteriosclerosis. No atheromatous degeneration or ulceration is noted. The internal mammary arteries send off branches to the anterior aortic intercostals and communicate freely with the inferior epigastric arteries. The major part of the collateral circulation appears to be between the superior intercostal, arising from the left subclavian artery and the first aortic intercostal (which springs from the aorta just below the second level of the coarctation), the posterior scapularis, the interscapularis, and the subscapularis which, piercing the intercostal spaces from behind, anastomose with the second, third and fourth aortic intercostals.

The superior vena cava is completely occluded from its origin in the right ventricle to its termination in the right jugular and the right subclavian veins (Fig. 3). This obliterative process includes the right innominate vein and one-half of the left innominate vein. The azygos vein is of moderate size and is not dilated. It terminates in the superior vena cava within the area of occlusion. The hemiazygos shows no dilatation. The azygos vein anastomoses freely at its caudal end with the inferior vena cava by way of branches

from the right renal vein and the inferior vena cava. The superficial veins of the thorax and abdomen are considerably dilated and display extensive anastomoses. There is noted a dilatation of the superficial circumflex iliacs, superficial epigastrics, axillary and costo-axillary, mammary venous plexus and thoraco-epigastric veins. There is also considerable dilatation of the deep epigastric, internal mammary, subclavian, intercostal and lateral thoracic veins.

### MICROSCOPIC EXAMINATION

Microscopic examination of various organs reveals a fibrocaceous tuberculosis of the right suprarenal gland; advanced arteriosclerotic nephropathy; lobar pneumonia in the stage of gray hepatization affecting the lower lobe of the left lung, with bronchopneumonia of the upper lobe; papilloma of the urinary bladder with early carcinomatous changes; diffuse pseudomembranous cystitis; focal purulent prostatitis; patchy myofibrosis of the heart; arteriosclerosis of the aorta; and chronic passive hyperemia of the liver and spleen. Sections of the cord-like superior vena cava show a large amount of hyalinized fibrous tissue containing small vascular channels, some of which are blocked by freshly formed thrombi.

*Anatomical Pathological Diagnoses:* Double coarctation of the aorta, infantile and adult types, the latter producing pronounced stenosis; moderate dilatation of the ascending aorta with beginning saccular aneurysm; extreme dilatation and tortuosity of both subclavian, internal mammary, first aortic intercostal, posterior scapularis, interscapularis, and subscapularis arteries proximal to coarctation, and of the first four pairs of intercostals, deep epigastrics and circumflex iliac arteries distal to coarctation; fusiform aneurysmal dilatation of thoracic aorta immediately caudal to second coarctation; erosion of intercostal grooves of the upper ribs; probable congenital bicuspid aortic valve with partial calcification and stenosis; subaortic annular calcification ring extending into bicuspid aortic valve; healed obliterative pericarditis; hypertrophy and dilatation of the heart; cor pulmonale; pronounced coronary arteriosclerosis; diffuse patchy myofibrosis of the heart; moderate generalized atherosclerosis; pronounced pulmonary arteriosclerosis; old thrombotic occlusion of the superior vena cava and innominate veins; pronounced dilatation of the superficial and deep veins of the abdomen and thorax, lateral thoracic, intercostal, internal mam-



mary, subclavian, and the epigastric veins; slight dilatation of the internal hemorrhoidal venous plexus; lobar pneumonia, left lower lobe; bronchopneumonia, lower right and upper left lobes; acute bronchitis, left; healed obliterative pleuritis, bilateral; calcified empyema pocket at the posterior bases of the pleural cavities; high grade arteriosclerotic nephropathy, especially of the right kidney; calcified tuberculous tracheobronchial lymph nodes; multiple (five) traction diverticula of the esophagus; old fibrocaseous tuberculosis of the right adrenal; focal purulent prostatitis; papilloma of urinary bladder with early carcinomatous changes; diffuse pseudomembranous cystitis; chronic passive congestion of the viscera; and beginning postsacral decubitus ulcer.

#### DISCUSSION AND CONCLUSIONS

While conforming to the usual picture of aortic coarctation in respect to age, sex, bicuspid aortic valve, development of collateral circulation and notching of the ribs, the case herein reported differs sharply from nearly all others heretofore studied in that there occurs both an infantile and an adult type of coarctation. In one of Abbott's<sup>20</sup> papers is an illustration bearing the title "Double Coarctation of the Aorta," but neither in this paper nor in her review<sup>1</sup> of 200 cases is there any mention of concurrent infantile and adult coarctation. From this it may be inferred that Abbott failed to find any such cases in addition to the one observed by herself. Among the cases of coarctation reported since Abbott's<sup>1</sup> review in 1928 we have encountered only 3<sup>10, 20, 36</sup> that may be classed as double coarctation. The coexistence of infantile and adult coarctation lends support to the hypothesis of Craigie<sup>48</sup> that the basis of the anomaly lies in an embryological disturbance of formation and involution of the primitive aortic arch.

Another remarkable finding that makes the case reported here unique in the group of aortic coarctation is the concomitant occlusion of the superior vena cava and its innominate tributaries, necessitating the development of a venous collateral return for the head, neck, upper extremities and thorax fully as extensive and intricate as the aortic coarctation demanded of the arterial circulation in order that the abdomen, lower extremities and most of the thorax might receive arterial blood. Thus, of necessity, over the torso both the arterial and the venous blood flowed caudally and in parallel

vessels. There is nothing in the clinical history to indicate when the vena cava became occluded and the only possible clue as to the cause of the fully organized and canalized thrombosis of this vessel is that in conjunction with the pericarditis or pleuritis there may have been a thrombophlebitis of the vena cava superior and its tributaries.

Contributing to the final cardiac decompensation was not only the coarctation of the aorta but the healed pericarditis and pleuritis binding the contents of the thorax to the chest wall and the diaphragm, thus throwing an additional load on the already burdened heart.

Still another cardiac complication deserves mention. In conjunction with the bicuspid aortic valve was a calcified ring which may be classed as a subaortic stenosis, an uncommon yet clinically important cardiac lesion. We are unable to say whether the calcification is part of a degenerative process or the end result of a healed valvulitis, but are inclined to favor the first possibility.

The case reported here illustrates in a remarkable manner the ability of the circulatory system to compensate for obstructions of even major vessels, and further, the reserve strength of the heart which withstood not only the coarctation but also, prior to the last attack, the toxic effects of infection (pneumonia and empyema) and carried well the increased load occasioned by the adhesive pericarditis and pleuritis for an indefinite period of time.

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## DESCRIPTION OF PLATES

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### PLATE 52

FIG. 1. Actual size drawing of aorta and certain of its branches: 'a' = greatly enlarged left subclavian artery; 'b' = lumen of aortic arch; 'c' = infantile coarctation; 'd' = diaphragm-like adult type coarctation (cut off-center) with only the tiny opening shown on the under surface. Between the two points of stenosis is a sacculated segment of aorta. On the right (pulled back) are the enlarged first four pairs of intercostal arteries.

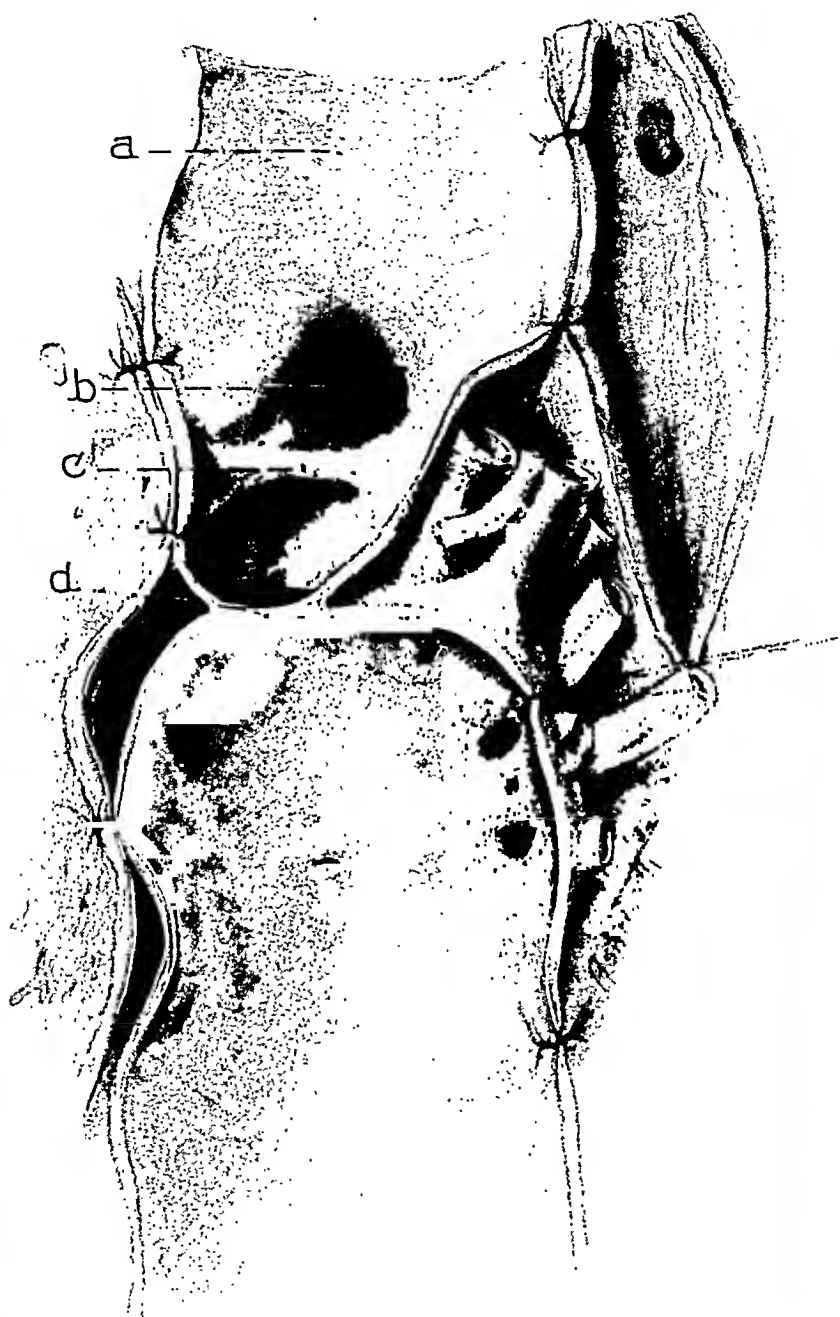
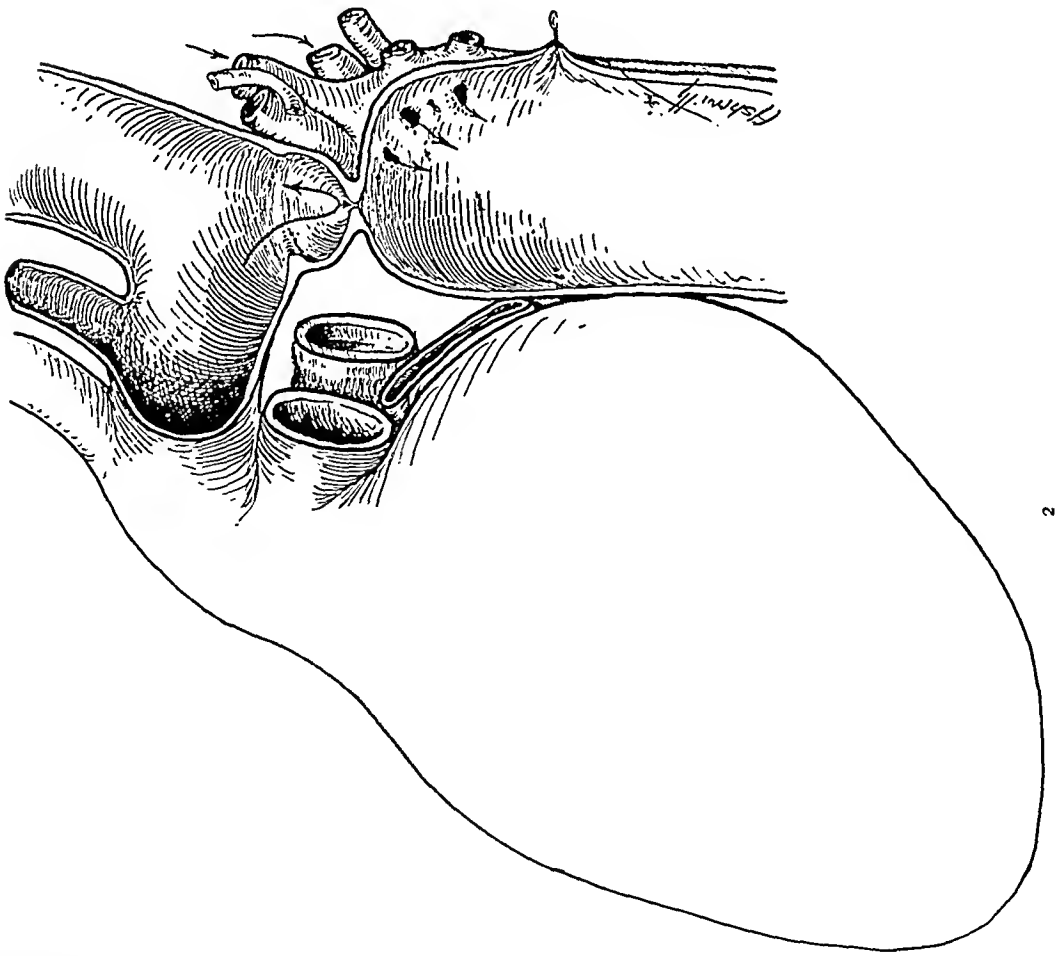
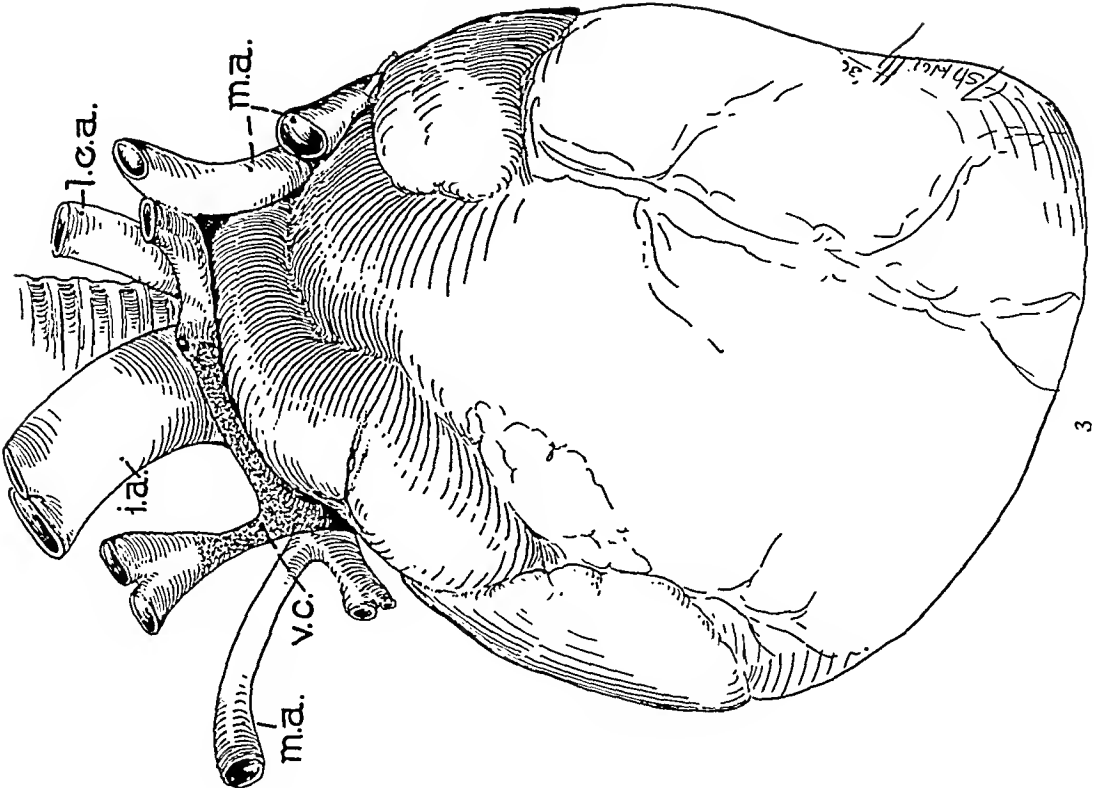


PLATE 53

FIG. 2. Diagrammatic drawing illustrating location of coarctations, direction of flow of part of the arterial blood, and comparative size of left common carotid and left subclavian arteries (opened). Compare with Fig. 1.

FIG. 3. Shown diagrammatically are the occluded superior vena cava (v.c.) and its innominate tributaries, innominate (i.a.), left common carotid (l.c.a.) and internal mammary (m.a.) arteries, prominent conus arteriosus and enlarged heart.







## CHRONIC DIFFUSE MESAORTITIS\*

### REPORT OF TWO CASES OF UNUSUAL TYPE

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It is our purpose to describe the pathology of 2 cases of chronic diffuse mesaortitis in which the aorta presented microscopic changes unlike any hitherto observed in this department or recorded in the available literature.

### CASE REPORTS

CASE 1. A. L., P. H. No. 442,294, autopsy No. 11,750, a Jewish male 76 years of age, born in Austria. The past history was incomplete regarding infections, diseases and habits. The illness for which he was admitted to the hospital centered about increasing difficulty in urination over a period of 3 years because of an enlarged prostate. A bilateral, direct inguinal hernia without complications had been present for many years. No symptoms referable to the cardiovascular system were obtained.

*Physical Examination:* The temperature, pulse rate and respirations were normal. The blood pressure was 168/80. There were extensive dental caries and pyorrhea alveolaris. The lungs were clear. The area of cardiac dullness was of normal outline; the sounds were regular and of good quality. Reducible direct inguinal herniae were present. The prostate was considerably enlarged, smooth, firm, mobile and non-tender. There were no other abnormal findings. No evidence of cardiac failure was noted.

*Laboratory Data:* The hemoglobin was 85 per cent, the red blood count 4,600,000. The white cell count was 13,000 with 85 per cent polymorphonuclear leukocytes. The blood Kahn and Wassermann reactions were negative. The blood urea was 20.3 mg. per 100 cc.; the blood sugar 111 gm. per liter. The urine showed a heavy trace of albumin with numerous red cells and an occasional white cell. Electrocardiogram revealed an occasional premature beat arising in the node but there was no indication of heart muscle damage.

X-ray of the chest demonstrated no abnormality of lungs or heart. The aorta appeared tortuous. There was an irregular shadow opposite the third and fourth ribs on the left side, which was interpreted as a diffuse dilatation of the arch of the aorta. X-rays of the urinary tract disclosed no abnormality.

After a vasectomy and a period of 2 weeks with indwelling catheter, a transurethral fulguration of the prostate and neck of the bladder was performed. The temperature rose steadily after this procedure and the patient died 5 days later with signs of consolidation of the lungs.

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*Postmortem Examination*

Autopsy was performed within 1 hour after death. An acute inflammation of the bladder with numerous suppurative foci in the kidneys, thought to be of recent hematogenous origin, were the principal findings. A considerable portion of the prostate remained. It included many nodules of hypertrophic tissue but no evidence of a malignant tumor. No areas of suppuration were present. There was also an extensive confluent lobular pneumonia in both lungs. The heart weighed 310 gm. and presented no abnormalities. The aortic ring measured 7 cm. and the valve appeared delicate but competent.

The aorta was somewhat tortuous as it lay in the body and had lost a considerable part of its elasticity. The approximate circumference after fixation was: ascending aorta 8.1 cm., midportion of the arch 6.6 cm., midthoracic region 5.9 cm., and midabdominal region 3.9 cm. The average normal measurements of the circumference of the aorta in these locations in males between the ages of 71 and 80 years are recorded by Roessle and Roulet<sup>1</sup> as 8.2 cm., 6.7 cm., 5.84 cm., and 4.7 cm. respectively. This aorta, then, could not be considered dilated at any point.

There was a slight diffuse intimal thickening, such as is customarily found at this age, augmented by numerous, irregular, bright yellow zones of poor definition, as well as the usual firm, opaque gray raised plaques which so frequently encircle the orifices of the intercostal and lumbar arteries. Small zones of intimal calcification were sometimes found in the plaques but were nowhere prominent. These changes were more intense in the lower thoracic region than elsewhere. A fine longitudinal wrinkling of the intima was also noted within and bordering the plaques throughout the length of the vessel. No ulceration was present. All of these findings differed in no way from the usual lesions of arteriosclerosis and they were thus so interpreted. They were also found in all of the large arteries throughout the regions of the body examined.

The cross-section of the aorta revealed no changes of note. The media was quite irregular in thickness but not more so than one would expect with the sclerotic lesions. It could not be split apart at any point and no vascular invasion was apparent. The adventitia was not thickened. The gross appearance of the aorta, then, was not unusual in an individual of this age.

*Microscopic Examination*

After random sections showed a curious alteration of the media, microscopic study was made of the entire length of the aorta. This was done by cutting two long strips, including half the length of the vessel in each and coiling them until they could be accommodated on the ordinary slide. They traversed many intimal plaques formed by hyalinized connective tissue or collections of fat-laden mononuclear cells typical of arteriosclerosis.

The media presented a variegated appearance. At intervals the fibrillar structure was in good part obscured by a diffuse and pronounced cellular infiltration. The great majority of the cells were small lymphocytes, but plasma cells (studied with the orcein-Giemsa and methyl green-pyronin stains) and an occasional polymorphonuclear leukocyte were present as well. An unusual finding was the presence of several multinucleated giant cells of the foreign body type sometimes surrounding refractive fragments which in some instances stained as elastic fibers. None of these cells showed a predilection for the perivascular zones nor were they any more numerous in the outer or the inner portion of the media.

In the areas so involved the fate of the fibers could not be determined with the hematoxylin and eosin stain. A combination of the Weigert elastic tissue and Van Gieson connective tissue stains showed a surprisingly mild degree of destruction. The musculo-elastic layer was composed of delicate fibrils and was often indistinct. In the inner third of the media the elastic fibers were regularly disposed and no depletion was noted. In the middle and outer thirds, but particularly in the former, there was a moderate thinning out of elastic, muscle and collagen fibers, but only small areas completely devoid of elastic tissue. The defect was not occupied by collagenous tissue, as is so often the case in luetic aortitis, and there was no proliferation of capillaries to invade the infiltrated portions of the wall. No fat was demonstrated in the media by scharlach R staining. There was no edema.

The adventitia was quite normal, failing to show any thickening or obliterative changes in the nutrient vessels. Occasionally small collections of lymphocytes were present, but not often associated with arterioles.

These changes were found at irregular intervals in both thoracic

and abdominal portions of the aorta and could not be correlated with the degree of intimal sclerosis. Where the cellular infiltration was absent or minimal, the continuity of all medial fibers was preserved, but there was sometimes a mucinous degeneration as shown by the mucicarmine stain. Fine granules identified as calcium salts by the von Kossa stain were disseminated throughout the entire medial coat. The amount of mucinous material and calcium salts was no greater than is observed in the majority of aortas in this age group (Paige and Mott<sup>2</sup>).

No organisms of any description could be demonstrated with the Gram, methylene blue, carbol fuchsin or Levaditi stains.

Sections through the carotid, pulmonary, renal and coeliac arteries failed to reveal similar infiltrations but a reaction quite like that in the aorta was found in the common iliac arteries. Since the lesion was not suspected at the time of autopsy, studies of other arteries could not be made. The medium and small arteries of the viscera were normal.

CASE 2. H. O'C., P. H. No. 439,569, autopsy No. 11,935, a white male, 50 years of age, of non-Jewish heritage, born in Canada and regularly employed as a carpenter. Thirty-five years before his entrance into the hospital he had two attacks of malaria, for which he received quinine. No recurrences were recognized. Typhoid fever, pneumonia, scarlet fever, diphtheria, rheumatic fever and venereal infection were denied. The consumption of alcohol was said to have been slight. No drugs were taken and smoking was confined to a pipe conservatively used. No dietary peculiarities were noted.

The present illness was essentially one of cardiac failure with recurring attacks of precordial pain over a period of 1 year. These were sufficiently severe to be prostrating at times, and toward the latter part of his illness pulmonary edema was pronounced. Medication included luminal, codein, cod liver oil, nitroglycerin, aspirin and ferrous carbonate in the usual dosages.

*Physical Examination:* When first admitted the temperature, pulse and respiration rates were normal. The blood pressure never became elevated. An average reading was 120/70. The lungs were clear in the earlier stages of the illness but later showed signs interpreted as edema. The heart became considerably enlarged and a friction rub was heard. The lower border of the liver was felt just below the costal margin.

*Laboratory Data:* The hemoglobin and red cell count remained within normal range. The white cell count varied from 10,000 to 16,000 with a normal partition. The usual urine and stool examinations were negative. The blood Wassermann reaction was negative. X-rays of the spine were obtained because of the complaint of muscle pains and hypertrophic changes consistent with the diagnosis of rheumatoid arthritis were demonstrated. In this connection the patient was found to have antistreptolysin in the blood, active in a dilution of 1:320. The electrocardiogram suggested considerable myocardial damage. There was a left ventricular preponderance.

The course of illness was one of improvement with rest, although there were numerous recurrences of cardiac and respiratory distress. Shortly before death the temperature rose to 103° F.

### *Postmortem Examination*

Autopsy performed 7½ hours after death disclosed advanced arteriosclerosis of the coronary arteries with occlusion of both branches of the left artery by fibrous tissue and yellow plaques. Extensive recent infarction involved the wall of the left ventricle and the large amount of scar tissue present bore witness to a vascular change of long standing. The heart weighed 460 gm. The aortic ring measured 7 cm. Incidental findings included a bilateral lobular pneumonia, arteriosclerotic scars in the kidneys and hyalinization of a few of the pancreatic islands of Langerhans.

Interest again centered about the changes in the aorta which closely simulated those in the previous case. No tortuosity was noted but the elasticity was patently reduced. There was no dilatation. The circumference after fixation was: ascending aorta 7.6 cm., midportion of the arch 6.4 cm., midthoracic region 5 cm., and mid-abdominal region 4.4 cm. The intimal changes were minimal but bright yellow, slightly raised plaques and gray fibrous areas of thickening were present, predominantly in the abdominal aorta. Calcification was found only at the site of the obliterated ductus arteriosus and there was no ulceration. On close inspection fine linear wrinkling could be discovered in three small areas, two above and one below the level of the diaphragm.

On section the media appeared to be quite regular and normal in width and no gross degeneration was noted. The vasa vasorum were not prominent in the medial coat. The adventitia was not thickened.

### *Microscopic Examination*

The microscopic appearance of the aorta was even more striking than in the preceding case. Similar stains were employed in the study of cells and fibers and sections were made which included the entire length of the vessel as described above.

The intima was either of normal width or thickened by fibrous tissue showing occasional degeneration, such as is seen in the deeper portions of arteriosclerotic plaques. The media was not reduced in width at any point. The cellular infiltration was diffuse and almost

uniform throughout the length of the aorta. Plasma cells were relatively more numerous and multinucleated giant cells less conspicuous than in the previous case. The latter cells often contained refractive bits of material which sometimes took the stain for elastic tissue. On the whole, there was no increase in the vascularity of the media but at times small vessels of capillary caliber penetrated the entire width of this coat.

Although the cellular infiltration was more pronounced than in the previous case, degeneration of elastic fibers was either only moderate or entirely absent. Where present it occurred in any part of the media but more often was found in the middle third. The intact elastic fibers were moderately wavy and regularly disposed. At no point was scar tissue found in the media, even in areas where there was some depletion of elastic fibers. The adventitia was uniform in thickness. There was no additional collagenous tissue and changes in the nutrient vessels could not be found.

A moderate cellular infiltration was found in the iliac and carotid arteries, but the renal and pulmonary arteries were normal. The left coronary artery was occluded by fibrous tissue thickening of the intima and the media showed only the reduction in width customary in such lesions. The visceral arteries were not significantly altered.

### DISCUSSION

The aortic lesion encountered in these 2 cases is a chronic inflammatory infiltration of the media with minimal degeneration. The intima and adventitia were not involved and no changes could be detected that seemed to be dependent on the medial reaction. Although the cellular invasion was extensive and most striking, the gross alterations of the aorta were insufficient to allow recognition of the lesion without microscopic study. The loss of elasticity can scarcely be ascribed to the medial changes since this is a more common finding in elderly persons. Although dilatation of the aorta was suspected in 1 case by X-ray, this observation was not corroborated by actual measurements of the vessel.

There would seem to be no clinical manifestations of this aortic lesion. No facts in the past history were ascertained suggesting a previous acute infection in the aorta. In the 1st case clinical observers were totally unaware of any damage to the cardiovascular system. The 2nd case presented the picture of progressive

cardiac failure, but this could be referred entirely to sclerosis of the coronary arteries and myocardial infarction. The blood pressure was not elevated in either case. Unfortunately readings were taken only on the upper extremities so that no information is at hand concerning the maintenance of the pressure throughout the entire aorta.

In neither case was there clinical evidence that an infection was present in the body, except as terminal complications of pneumonia and suppurative nephritis. The temperature of each patient was normal on admission. No abnormal cells appeared in the blood, although 1 case, with infarction of the heart, persistently showed a slightly elevated white blood cell count. The erythrocyte sedimentation rate was moderately elevated in the case in which myocardial infarction was present. It was not recorded in the other case.

No clue as to the etiological factor has been gained by morphological studies or by review of the literature. Degenerative and inflammatory changes in the aorta caused by a variety of agents have been described. They are all sufficiently unlike those observed in our 2 cases to justify placing the latter in a distinct category.

The lesion is readily distinguished from the ordinary medial necrosis of the aorta described by Erdheim<sup>3</sup> and others. The typical degeneration classified under this group shows an alteration of the interstitial tissue with the production of a basophilic substance, staining like mucin, and in the more advanced cases depletion of fibers, cystic formation or longitudinal splitting through the wall. There need be little scar tissue and the intima and adventitia are not involved, features which liken the Erdheim necrosis to the lesions in these 2 cases. However, the difference in degree and character of the cellular infiltration is a striking one, and in the cases reported here the degeneration is so mild that it is largely obscured by the massive accumulation of inflammatory cells in the media.

The lesion is decidedly reminiscent of a syphilitic aortitis but there are many factors in which the two conditions are quite dissimilar. In these 2 cases no significant changes could be detected on macroscopic examination, even after the nature of the medial infiltration was known. The intima presented none of the diffuse irregular thickening and deep crevices associated with a luetic infection. Furthermore, alterations were found in various parts of the



aorta and were not limited to the thoracic portion of the vessel, as is more often the case in lues. While destruction of elastic fibers in the media did appear at intervals, the areas were not converted into scar tissue and vascularization of the media was not a feature of any prominence. The types of cells invading the wall are in accord with those found in syphilis, but in the latter disease they frequently form collars about the vasa vasorum and are rarely so uniformly disseminated throughout the entire media. Giant cells have accompanied gummatous degeneration of the aorta but no necrosis was present in conjunction with the large cells in these 2 cases. The appearance of the adventitia was not that of a luetic aortitis since at no point was there thickening, obliterative changes in the nutrient vessels, or perivascular accumulation of cells. As corroborative evidence, the Wassermann reaction in each individual was negative.

Rheumatic aortitis has been described in a variety of forms (Klotz,<sup>4</sup> and Pappenheimer and VonGlahn<sup>5</sup>), none of which is simulated by the lesion here discussed. The cellular infiltration of the intima and subjacent positions of the media with palisades of deeply staining basophilic cells with distorted nuclei, as seen in rheumatic aortitis, was entirely lacking. Adventitial sclerosis and accumulation of typical cells were not found. Other rheumatic lesions were lacking in each case and the history suggested in no way that either individual had suffered from this disease.

Tuberculous foci in the media of the aorta have been present in the form of miliary tubercles or, more rarely, as caseous areas, but no lesion so extensive has been ascribed to the tubercle bacillus. The giant cells present in our cases were not those of Langhans but were of foreign body type, and there was not the slightest suggestion of tubercle formation. Repeated staining with carbol fuchsin failed to reveal any acid-fast bacilli.

A variety of mycotic infections can conceivably stimulate such a cellular response but no fungi could be identified in the wall of the aorta or in any of the viscera.

It has occurred to several pathologists studying these cases that they may represent the chronic phase of an infectious process such as that repeatedly described in the literature as acute aortitis. Influenza is sometimes accompanied by arterial thrombosis, thought to be secondary to bacterial invasion of the wall. The lesion described by Marmorstein<sup>6</sup> in a young female dead of influenza was confined to

the aortic intima over an area about 1.5 cm. in diameter and could be in no way related to the lesion under discussion. Kuskow<sup>7</sup> describes only endothelial changes associated with influenzal infections.

The streptococcus has received considerable attention in relation to alterations in the arterial system, both as isolated case studies and in animal experimentation. Clawson<sup>8</sup> obtained an arteritis, involving all coats, by injecting *Streptococcus viridans* from the blood of patients with rheumatic fever into rabbits and monkeys. The changes were likened to the rheumatic lesions in man and were not those of a possible forerunner of the lesions found in these 2 cases. Benson *et al.*,<sup>9</sup> on the other hand, produced thickening and scarring of the intima, and a mild cellular infiltration of the adventitia and media of rabbit aortas with injections of streptococci, but the lesions were not suggestive of those described above.

The streptococcus is of particular interest since in 1 of the cases a high antistreptolysin titer of the blood was present and a clinical diagnosis of rheumatoid arthritis was made. There have been numerous case reports of focal purulent response in the aorta associated with systemic streptococcal infection, usually occurring in a single isolated area and thought to follow the introduction of organisms via the vasa vasorum or by means of septic emboli lodged on the intimal surface. We are unable to find a reported instance of diffuse phlegmonous mesarteritis in an acute streptococcal disease.

Brody and Smith<sup>10</sup> reviewed the pathology of 44 cases of scarlet fever and 15 cases of possible scarlet fever with the finding of aortic changes in 1 case alone. The capillaries of the adventitia of the aorta are described as having a border of mononuclear cells. No medial infiltration was noted.

The effect of diphtheria toxin on the arterial system of the rabbit has been investigated and reviewed by Duff,<sup>11</sup> who found a purely degenerative lesion in the aorta unaccompanied by inflammatory change. There was occasional calcification and he likens the lesion to the Mönckeberg sclerosis in man.

An acute arteritis occurring in typhoid fever has frequently been observed, with thrombosis and subsequent gangrene of extremities. The extensive literature on this subject is presented by Ophüls.<sup>12</sup> The affected artery is almost always a small one and a pronounced infiltration of the aorta is not described. MacCallum,<sup>13</sup> in discussing the rôle of infections in the pathogenesis of arteriosclerosis, describes

the presence of atheromatous deposits in the intima in a large proportion of typhoid cases in youth but does not record inflammatory changes in the media.

The possible later developments in typhoid fever were investigated by Thayer,<sup>14</sup> who concluded that arterial thrombosis was present because of an underlying arteritis, but this statement is not substantiated by histological studies.

Pneumonia caused by the pneumococcus has been accompanied by acute infections in the arteries, either in the form of small pyogenic foci in the aorta, or in the smaller arteries with thrombosis (McGregor,<sup>15</sup> and Ophüls<sup>12</sup>). The gonococcus has also been present in circumscribed acute infections of the aorta (His<sup>16</sup>), but has never been found with extensive involvement of the aorta.

Localized ulceration, gangrene and perforation of the aorta following infection by *B. anthracis* were described in a single case report (Oliver<sup>17</sup>). In our own laboratory *B. pyocyaneus* was responsible for an acute secondary involvement of a calcareous aortic plaque and the media beneath this plaque was moderately infiltrated with polymorphonuclear leukocytes; there was no inflammatory reaction elsewhere in the aorta (Fish, Hand and Keim<sup>18</sup>).

A review of the autopsy findings in a large variety of infectious diseases by Wiesel<sup>19</sup> includes aortic lesions, notably medial necrosis and edema, but no cellular infiltration accompanied the degeneration and the lesions were usually circumscribed.

Lacking evidence that organisms throughout the media of the aorta initiated the process, which we found as a chronic inflammation and mild degeneration, it is inviting to consider the possibility that a purely allergic reaction is responsible for the entire change. Seegal *et al.*<sup>20</sup> described an intense inflammatory reaction in the pericardium, myocardium and intrapericardial portion of the aorta in sensitized rabbits following intrapericardial injection of the homologous antigen. The aorta in most instances showed some edema, hemorrhage and cellular infiltration in the adventitia. There were also subendothelial collections of polymorphonuclear leukocytes which, in 1 case, extended through the inner half of the wall. When the animals were allowed to live longer after the injection, the polymorphonuclear leukocytes were largely replaced by lymphocytes and mononuclear cells. No organisms were found.

The objections to such a theory, when applied to our cases, are

patent. The Arthus phenomenon, as described by many, is an intense local response at the site of the injection and it is difficult to conceive of a possible portal of entry of a specific antigen which would extensively damage the media of the aorta without intimal or adventitial injury. Furthermore, no positive information regarding a mode of sensitization can be gained from a review of the history in either case. Such an interpretation would be based on pure supposition.

There are numerous chemical substances that are known to affect the aorta in animals. Caution must be used in interpretation of results obtained by experimentation, since they may be quite unique and not at all comparable to the effect of the toxic substances in man. Saltykow<sup>21</sup> describes the lesions produced in rabbits with epinephrin by several investigators. They are usually confined to the media and are characterized by necrosis, scarring and calcification. Again, inflammatory changes are minimal. Saltykow also reviews the work of Fischer who injected hydrochloric acid, phosphoric acid, lactic acid, calcium phosphate, potassium bichromate, uranyl nitrite, chloralamide, mercuric chloride, phlorizine, trypsin, pepsin, iodothyrene, mamma siccata and sodium chloride, obtaining medial lesions but less regularly and not of such a notable character as with epinephrin. The calcification of the media is again a prominent feature and no mention is made of cellular response.

Uranium was found to exert a similar effect by Dominguez,<sup>22</sup> when injected alone or with lead, radium or vanadium, but the latter alone were inert as far as arterial lesions are concerned. The effects again consisted of medial calcification and were quite distinct from the type of response in our 2 cases.

The extensive literature on intoxication by heavy metals, alcohols, tobacco, the halogens, coal tar derivatives and radioactive substances was searched for the production of a medial infiltration or degenerative change in the aorta, without success. The lesion is not reported in man or animal associated with the toxic substances investigated.

#### SUMMARY

Two cases presenting diffuse changes in the media of the aorta which do not conform to any recognized type of aortitis are described. They consisted briefly in extensive cellular invasion of a

chronic inflammatory nature with little degeneration of the elastic fibers and no scar formation. The intima and adventitia were not involved. Bacteria were not demonstrated. The medial alteration produced no characteristic changes in the aorta that could be recognized on gross examination. There were no demonstrable clinical manifestations. The histories yielded no clue as to the etiological agent. The literature on medial changes in the aorta is reviewed. No comparable lesion was found.

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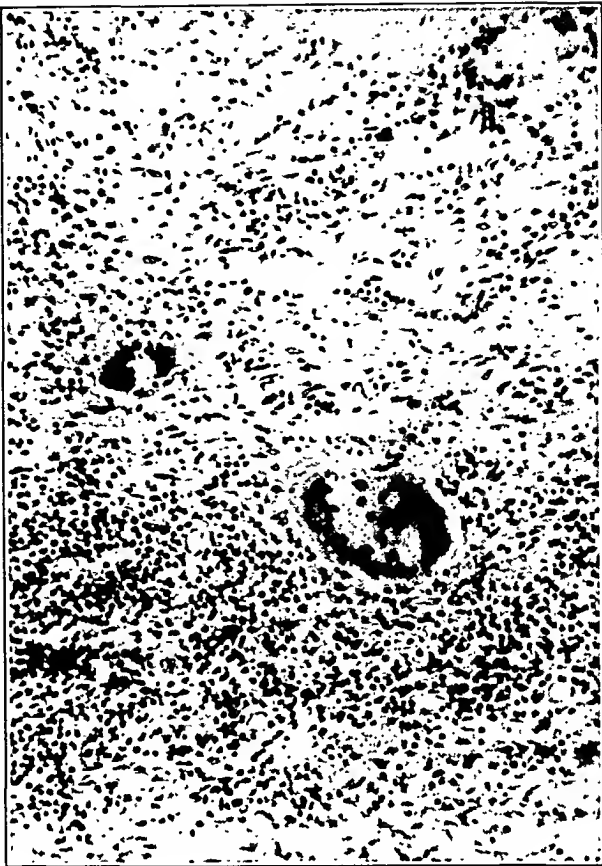
## DESCRIPTION OF PLATES

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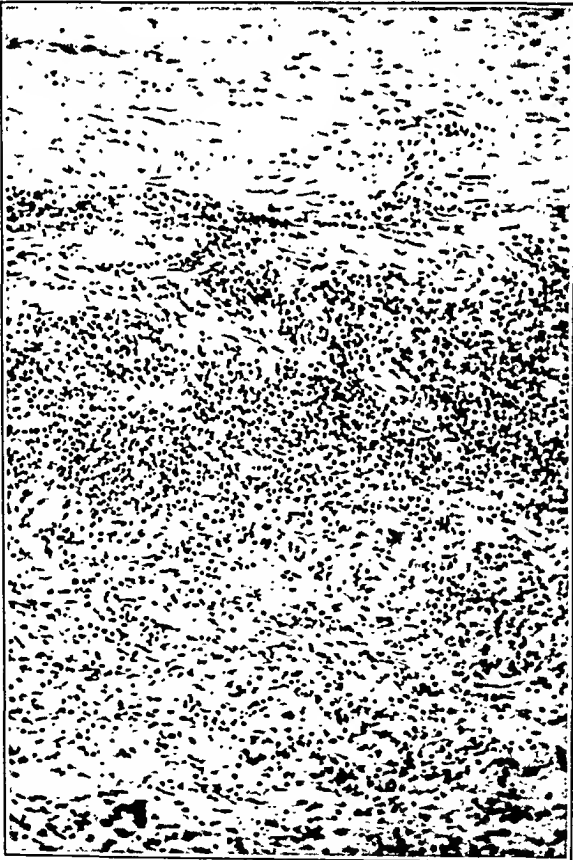
### PLATE 54

FIG. 1. Case 1. Diffuse infiltration of the media of the aorta, showing two foreign body giant cells.  $\times 170$ .

FIG. 2. Case 2. Similar diffuse infiltration of the aortic media.  $\times 110$ .



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## EFFECT OF PARATHYROID EXTRACT AND CALCIFEROL ON THE TISSUES OF THE NEPHRECTOMIZED RAT \*

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Albright and Ellsworth<sup>1</sup> and Ellsworth<sup>2</sup> have developed the idea that the activity of the parathyroid glands is dependent on the excretory function of the kidney. Since increased phosphate excretion quickly follows administration of parathyroid extract they suggested that the outpouring of phosphorus into the urine is a primary hormone effect, which is followed by a resultant rise in serum calcium. Recently Ellsworth and Futcher<sup>3</sup> have reported that they have obtained a rise in the serum calcium of nephrectomized dogs after the injection of parathyroid extract. They have concluded that the elimination of phosphorus in increased amount by the kidneys is not necessary in the dog for the elevation in the serum calcium that occurs after the injection of a suitable dose of parathyroid extract.

Three years ago two of the authors<sup>4, 5, 6</sup> began an experimental study of calcium and phosphorus metabolism and of hormone action in nephrectomized rats and dogs. Our data indicate that the presence of functional kidney tissue is a requisite for the elevation of blood calcium that characterizes overdosage with parathyroid extract. While our experiments were in progress Collip and co-workers<sup>7</sup> reported an examination of the bones of 8 nephrectomized rats that had been injected with parathyroid extract. On the basis

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of the osteoclastic resorption observed they concluded, without chemical data, that hormone action was independent of kidney function and interpreted the evidence as a refutation of the hypothesis first developed by Albright and Ellsworth.<sup>1,2</sup> The present paper, in which tissue effects are described, is an effort to clarify and correlate some of the histological and chemical changes brought about by parathyroid hormone and vitamin D.

Since parathyroid bone lesions were described by Jaffé, Bodansky and Blair<sup>8</sup> numerous articles dealing with the skeletal changes that follow overdosage with both parathyroid hormone and vitamin D have appeared. Most workers agree that the bone lesions produced by these two substances are in certain respects dissimilar. Shelling,<sup>9</sup> using rats which weighed 50 gm., found that a chronic overdosage with viosterol gave decalcification without the fibrous replacement produced by parathyroid extract. Selye<sup>10</sup> likewise found fibrosis to be a prominent feature of chronic parathyroid hormone treatment. We were especially concerned with the production of acute lesions, and in acute experiments on 14 day old rats Selye observed a disappearance of bone trabeculae and the presence of osteoclasts as early as 20 hours after a dose of 10 units of parathyroid extract. Ham and Lewis<sup>11</sup> regard the osteoclast reaction as a change secondary to bone softening and state: "As a matter of fact the theory which postulates parathyroid hormone to act through osteoclasts is founded on a hypothesis which has been, for the most part, uncritically accepted for so many years that it has gained all the prestige of a fact." In our experiments with nephrectomized rats we have been much impressed with the value of the osteoclast reaction as an indicator of bone resorption.

## METHODS AND MATERIALS

The Wistar Institute albino rat was used. The experiments were acute, most of them terminating after 48 hours. Blood for the chemical analyses was withdrawn from the heart of the living rat without anesthesia. The calcium determinations were made by the Kramer-Tisdall method as modified by Tweedy and Koch.<sup>12</sup> In all instances calcium analyses were made on individual samples, consisting usually of 2 cc. of serum, but in a few instances of 1 cc.

## HISTOLOGICAL PROCEDURE

The femurs, fixed in 10 per cent formalin after decalcification for a minimum time in dilute nitric acid, were embedded in paraffin and the lower end of the femur sectioned in a plane to include the full curvature of the lower end of that bone. The descriptions and interpretations were made from an examination of the central portion of the metaphysis directly beneath the cartilage plate. In this location in the rats of the age used there are relatively few osteoclasts. This cell structure is readily and accurately identified and recognition of bone resorption was not attempted except in connection with osteoclast reaction. The osteoblastic component varies much with age and, since our examination was chiefly concerned with bone resorption, the osteoblastic variations have not been emphasized. After hormone administration spindle shaped cells (Fig. 1) appear and lend a peculiar appearance to the tissue. At certain stages of functional development of osteoblasts the structural differentiation of osteoblasts and fibroblasts is not easy and we have not attempted it.

Owing to the frequency with which lesions are produced by overdosage with parathyroid extract in the stomach (dog) and the heart (dog and rat), microscopic sections of the stomach were examined in many of the rats, and sections of the myocardiums of all animals were examined. Destructive lesions, necrosis, or hemorrhage with necrosis, were not found. In some animals necrotic or fibrotic foci were found in the heart muscle of rats that had been subjected to a prior heart puncture.

ACTION OF PARATHYROID HORMONE ON THE BILATERALLY  
NEPHRECTOMIZED RAT

Following operation the animals were not fed but were supplied with drinking water. It was found that fasting during a 48 hour period affected neither the calcium level nor the bone of the normal rat (Rats 1-3, Table I). In 9 rats that were bilaterally nephrectomized the serum calcium was within normal limits at the end of 48 hours, and the bone, on microscopic examination, was found to be normal (Rats 4-12, Table I). The remaining nephrectomized rats (Table I) were injected with parathyroid extract,\* and all showed

\* The parathyroid extract was generously supplied by Eli Lilly and Company.

characteristic bone changes (Fig. 1). In 5 out of 11 rats (Rats 13-23, Table I) the resorption was so extreme that spontaneous separation in the zone beneath the cartilage plate occurred during removal of the bone. The softening was accompanied by the increased osteoclastic reactions described by Collip and co-workers.<sup>7</sup> On the other hand the calcium, which averaged 12.36 mg. per cent in 4 of the animals, was not much changed.

TABLE I

*Effect of Parathyroid Extract on the Serum Calcium and on the Femurs of Bilaterally Nephrectomized Rats \**

Rat No.	Weight (individual or average)	Units parathyroid extract injected	Hrs. after sacrifice	Serum calcium	Femur
	gm.			mg./%	
1-3	89		48	12.06	Normal
4-12	151		48	10.94	Normal
13	107	250 x 4	48	13.64	
14	98	250 x 4	48	12.71	oc † + + + ob (spindle)
15	114	250 x 4	46		oc + + + ob (spindle)
16	111	250 x 3	26		oc + + + ob (spindle)
17	107	250 x 4	48		oc + + + ob (spindle)
18	127	250 x 4	48	10.48	oc + + + ob (spindle)
19	137	500 x 3	48	12.64	oc + + + ob (spindle)
20	107	250 x 3	26		oc + + + ob (spindle)
21	90	250 x 3	26		oc + + + ob (spindle)
22	90	250 x 3	26		oc + + + ob (spindle)
23	122	250 x 3	26		oc + + + ob (spindle)

\* Rats 1-3 were normal animals that were not fed for 48 hours. Rats 4-12 were nephrectomized, but not injected.

The potency of the parathyroid extract is expressed in "Hanson" units, one of which is one-fifth of a parathormone (Collip) unit.

† oc = osteoclasts with the degree of resorption and associated osteoclastic reaction indicated by +, ++, or +++. ob = spindle shaped cells (osteoblasts?).

It would appear that the calcium is removed from the bone into the extravascular soft tissues, where it is held, or that it is excreted by the intestines. Evidence of the former is lacking, but proof of colonic excretion is indicated. The calcium loosened from the skeletal storehouse does not accumulate in the blood. In other words, the usual criterion of parathyroid hormone action, namely the increase in blood calcium above normal levels, is lacking and the apparent inference made by Collip, Pugsley, Selye and Thomson<sup>7</sup> that bilateral nephrectomy does not influence hormone action is incorrect. Furthermore, the destructive lesions caused by overdosage of the normal animal with hormone were not observed by us in the ne-

phrectomized rat. Pugsley<sup>13</sup> has shown that the fecal excretion of calcium is increased following hormone administration, and we suspect that a greatly increased intestinal excretion, perhaps of the nature of a vicarious compensation, may rid the system of the calcium moved out of the bone. It has been established that the blood phosphorus rises rapidly after nephrectomy, and since the excretion of calcium may proceed more rapidly by the intestinal route than under normal conditions, these two conditions may affect the amount of calcium that accumulates in the blood.

#### ACTION OF CALCIFEROL ON THE BILATERALLY NEPHRECTOMIZED RAT

Calciferol \* administered in single subcutaneous doses to rats that were not fed during the 48 hour experimental period gave either doubtful or indecisive increases in the serum calcium (average 12.69 mg. per cent in Rats 1-4, Table II), and the bone was devoid of any distinct osteoclastic bone resorption. Even though large quantities of calciferol were injected subcutaneously in divided doses into normal rats (Rats 5-6, Table II) no osteoclastic reaction was produced within 48 hours. The remaining rats in Table II were bilaterally nephrectomized. Of these, Rats 7 and 8 showed osteoclast reaction to 1 cc. of calciferol (920,000 I.U.), but Rat 9 revealed no excess of these cells. There was no rise in calcium values above normal. Triple subcutaneous doses of 0.5 cc. of calciferol in Rats 16-17 gave definite elevations of calcium and a slight to a considerable osteoclast reaction with bone resorption (Fig. 3). The average amount of resorption and osteoclastic reaction in the series of calciferol-injected nephrectomized rats was not so great as in the nephrectomized animals injected with hormone, but in individuals of the former group the resorption and associated osteoclastic reactions were pronounced (Figs. 2 and 3). The "spontaneous" fractures frequently seen in the rats injected with parathyroid extract were unusual in the animals injected with calciferol. The calciferol injections in Rats 18-20 were delayed 5 to 20 hours after nephrectomy. Phosphorus determinations of the blood serum of these three animals showed an average terminal value of 24.35 mg. per cent, and yet the calcium was maintained at the normal value or slightly above.

\* The calciferol was generously supplied by Mead Johnson and Co.

## DISCUSSION

In a study of calcium and phosphorus metabolism an examination of both skeletal and soft tissues is useful for the reason that pathological quantities of calcium may become microscopically demonstrable in the form of calcification or resorption, but especially be-

TABLE II  
*Effect of Calciferol on the Serum Calcium and on the Femurs of Bilaterally Nephrectomized Rats \**

Rat No.	Weight (individual or average)	Calciferol solution	Serum calcium (individual or average)	Femur
	gm.	cc.	mg./%	
1-4	144	0.5	12.69	Normal
5	178	0.5 x 3		Normal
6	114	0.5 x 3	12.82	Normal
7	231	0.5 x 2	9.67	oc ++
8	239	0.5 x 2	10.23	oc ++
9	283	0.5 x 2	9.75	Normal
10	159	0.5 x 3	15.04	oc ++
11	144	0.5 x 3	15.77	oc ++
12	142	0.5 x 3	17.30	oc +
13	143	0.5 x 3	15.68	oc ++
14	129	0.5 x 3	16.04	oc +
15	150	0.5 x 3	13.22	oc ++
16	154	0.5 x 3	17.06	oc ++
17	159	0.5 x 3	15.04	oc ++
18	118	0.5 x 3	13.18	oc +
19	289	0.5 x 3	12.55	oc ++
20	180	0.5 x 3	11.40	oc ++

\* The calciferol was dissolved in corn oil and administered subcutaneously. Each 0.5 cc. contained 11.5 mg. calciferol and was equivalent to 460,000 international units of vitamin D. The multiple doses were spaced about 6-12 hours apart.

Rats 1-6 inclusive were not operated upon. In Rats 18, 19, and 20 the serum inorganic phosphorus was 23.86, 30, and 19.20 mg. per cent, respectively, at the end of 48 hours. All animals were sacrificed 48 hours after the operation.

cause cell reactions which are more or less characteristic may result from abnormal variations in the calcium and phosphorus content of the tissues. Of these cells the osteoclasts of the bone are often a conspicuous feature of resorptive processes. Not only is the origin of this cell in doubt but its function is uncertain. Many investigators regard the cell as the "bone" macrophage whose counterpart in the soft tissues is the common macrophage. Experimental evidence has shown that the latter produces at least one enzyme, a protease. We know of no proof that the osteoclast is the source of the calcium-

liberating property attributed to it by Collip and co-workers.<sup>7</sup> Neither do we see that Ham and Lewis<sup>11</sup> have shown that the osteoclast is not a potent factor in the type of resorption that is seen in the lesions that may be produced with parathyroid extract and calciferol. Regardless of what its function may be, the osteoclast furnishes important evidence of the presence or absence of the type of resorption that we are considering.

A comparison of parathyroid hormone and calciferol effects in the bilaterally nephrectomized rat reveals that although both the hormone and the calciferol loosen calcium and remove it from the bone, it is only calciferol that effects a hypercalcemia during our 48 hour experimental period. The explanation of this emphatic difference probably rests on the mechanisms involved. Brown and Shohl<sup>14</sup> and Watchorn<sup>15</sup> found that irradiated ergosterol decreased calcium excretion in the feces and increased urinary excretion. In a series of rats fed a calcium- and phosphorus-free diet Shelling<sup>16</sup> found that of the total calcium excreted 5 per cent was excreted in the urine and 95 per cent in the feces; while in another series of rats fed the same diet, except that large amounts of viosterol were added, he found that only 3 per cent of the calcium was excreted by the intestines and 97 per cent in the urine. Presumably in our bilaterally nephrectomized rats that were injected with calciferol the shutting off of all urinary excretion by removal of the kidneys, together with the calciferol action tending to lessen fecal excretion, accounts for the hypercalcemia observed. That both calcium and phosphorus may rise simultaneously to high levels in the nephrectomized animal is shown by our observations of nephrectomized rats,<sup>4</sup> and dogs<sup>5, 6</sup> that had been injected with calcium gluconate. In those experiments it was found that the rise in serum calcium was dependent on the introduction of a sufficient quantity of the calcium salt. In our series of calciferol-injected rats the calcium salts were drawn from the bone and the available evidence indicates that it accumulated in the blood because of decreased fecal excretion.

In the nephrectomized hormone-injected rat the situation is different. We think that it is quite probable that the amount of extraskeletal calcium liberated by the action of parathyroid hormone does not accumulate in the blood because much is eliminated by the intestinal route which is generally accepted to be the normal route. Pugsley,<sup>13</sup> using small doses of parathyroid extract, found an accen-



tuation of calcium excretion in the urine and feces. In the normal rat large subcutaneous doses of parathyroid extract are followed by hypercalcemia, although there is normal or excessive excretion of calcium by the intestines, but in the nephrectomized rat, in which there is a definite tendency for the phosphorus level to rise, and for the calcium to fall,<sup>4</sup> a characteristic hypercalcemia does not follow the administration of large doses of parathyroid extract.

### SUMMARY AND CONCLUSIONS

1. In the bilaterally nephrectomized rat the administration of large doses of parathyroid extract does not produce a characteristic rise in serum calcium but does cause a pronounced osteoclastic resorption of bone.

2. In the bilaterally nephrectomized rat subcutaneous injection of calciferol causes a hypercalcemia and also osteoclastic bone resorption within 48 hours.

3. The above observations are made the basis for a discussion of the probable mechanisms of action of parathyroid hormone and calciferol under the experimental conditions described.

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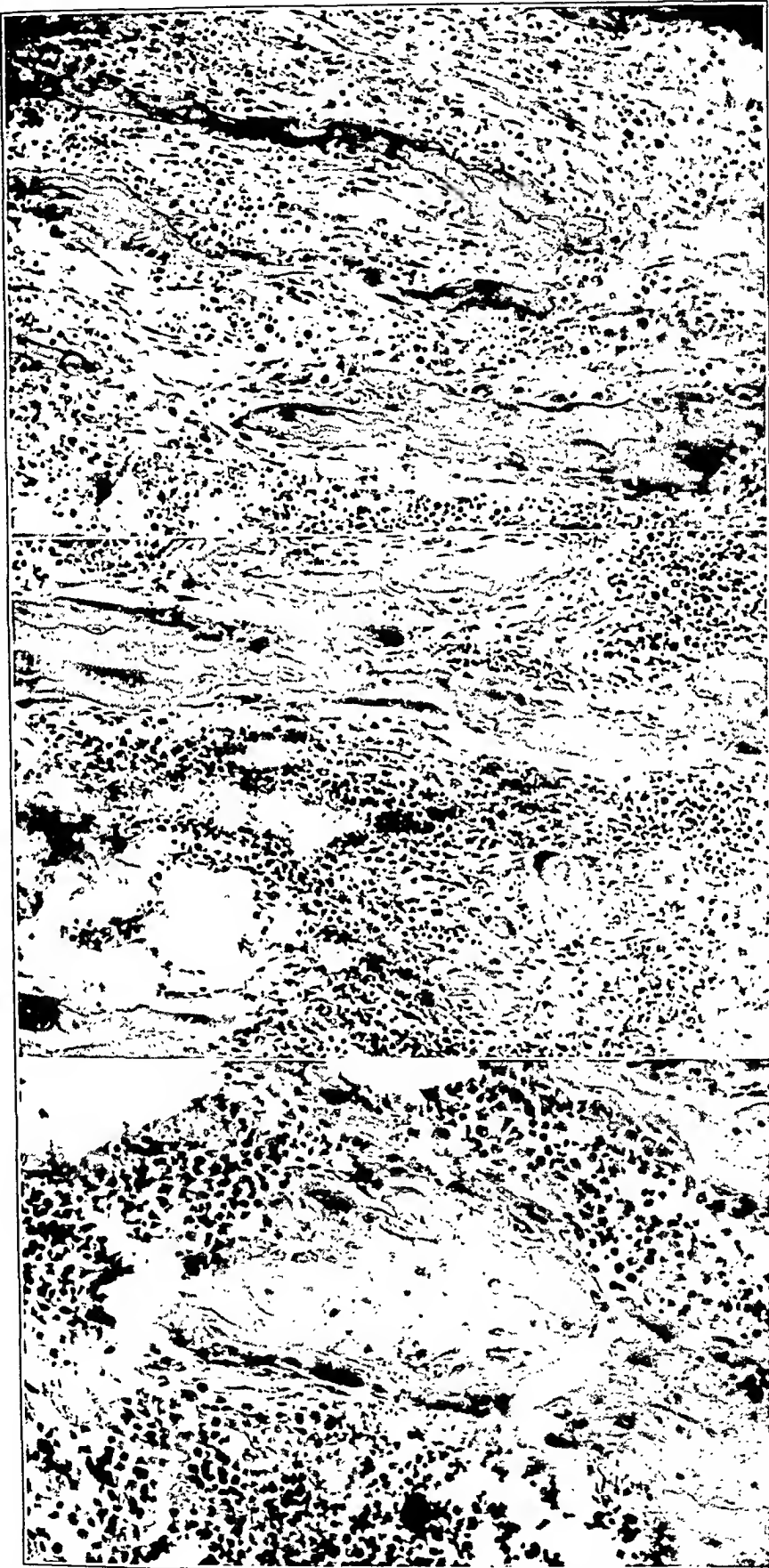
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## DESCRIPTION OF PLATE

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### PLATE 56

- FIG. 1. Photomicrograph from a section of metaphyseal end of femur showing resorption that followed in 26 hours after the injection of 750 (Hanson) units of parathyroid extract (Lilly) into nephrectomized Rat 16 (Table I). Numerous spindle shaped cells are apparent. Osteoclasts are present but not especially numerous.  $\times 141.4$ .
- FIG. 2. Photomicrograph from a section of metaphyseal end of femur showing the increased resorption of bone that followed in 48 hours after the administration of 1.5 cc. of calciferol ( $3 \times 460,000$  I.U.) to Rat 11 (Table II). Spindle shaped cells are less conspicuous than in the hormone-injected animal but osteoclasts are numerous.  $\times 141.4$ .
- FIG. 3. Photomicrograph from section of metaphyseal end of femur showing the increased resorption of bone that followed in 48 hours after the administration of 1.5 cc. of calciferol to nephrectomized Rat 17 (Table II). Here again the osteoclasts are active in the resorption.  $\times 258$ .



I

2

3



## PHAGOCYtic ACTIVITY OF CIRCULATING CELLS IN THE VARIOUS TYPES OF LEUKEMIA \*

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The purpose of this study was to determine the variations, if any, in the phagocytic activity of cells found circulating in the various forms of leukemia, as well as in normal blood, and thus to establish a physiological criterion for differentiation in addition to those well known morphologically. The work was carried on by the two workers on different cases at different institutions and employing differing methods.

### STRUMIA'S METHOD

The cells are obtained by taking 5 to 20 cc. of blood from the vein and mixing it immediately with 50 to 200 cc. of the following solution: sodium chloride 8.5 gm., sodium citrate 10 gm., and distilled water 1000 cc. By careful centrifugation the buffy layer or leukocytic cream is separated and washed three times with salt solution, the final suspension containing from 40,000 to 50,000 leukocytes per cmm.

In all the experiments pooled, fresh, unheated serum from adults was used. The bacteria were from a strain of *Staphylococcus aureus haemolyticus* that has been used extensively in the laboratory because it is readily phagocytozed. The cocci were obtained from an 18 hour broth culture and were washed three times with salt solution. The final suspension was made to contain two billion bacteria per cc.

One-tenth cc. of serum diluted with salt solution, 0.2 cc. of white cells in suspension, and 0.1 cc. of bacterial suspension are mixed in small glass tubes and shaken once a minute for  $\frac{1}{2}$  hour at room temperature. Immediately before smears are made 1 drop of fresh serum is added to each tube and the contents mixed. Smears are then prepared on clean slides, air dried and stained by a modification of the May-Grünwald-Giemsa method.<sup>1</sup> At least 100 cells of each type are counted, noting both the number of cells showing phagocytosis and the total number of bacteria contained.

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All the experiments on the phagocytic activity of circulating leukocytes were done over a period of 3 years, using exactly the same technic and thus obtaining comparable results.

Table I summarizes the results obtained in five experiments in which normal leukocytes from adults were employed. This table shows that maximum phagocytosis was obtained with neutrophilic polymorphonuclears, that the monocytes were a close second, although constantly less active than the neutrophilic polymorphonuclears, both in percentage of cells showing phagocytosis and in the total number of phagocytosed bacteria. The eosinophiles show distinctly less phagocytic activity than either neutrophils or monocytes, especially in the total number of ingested bacteria, and the lymphocytes show no phagocytosis at all.

### EXPERIMENTAL

*Acute Lymphatic Leukemia:* The cells were obtained from Margaret B., a white female, aged 14 years. At the time the cells were obtained the white count was 46,700 with 91.5 per cent lymphocytic cells, of which nearly one-half were lymphoblasts. The results in Table II show clearly that neither the immature lymphocytes (lymphoblasts, prolymphocytes) nor the mature lymphocytes showed any phagocytosis, and that the young and mature neutrophils showed phagocytic activity that may be considered close to the normal limits.

*Chronic Lymphatic Leukemia:* Mrs. Celia F., a white female, aged 60 years, presented a classical picture of the disease and at the time of the experiment showed 54,500 white cells per cmm. of blood, 96 per cent of which were lymphocytic, including 80.5 per cent prolymphocytes. None of the lymphocytic cells showed any phagocytic activity whatever, whereas the neutrophilic cells showed active phagocytosis (Table III). It may be stated here that in over 8 years of work on the problem of phagocytosis in which thousands of slides have been examined, phagocytosis by lymphocytic cells has never been observed, which seems remarkable if one considers that even accidentally bacteria might be expected to overlap occasionally on lymphocytes.

*Acute Myelogenous Leukemia:* Catherine R., a white female, aged 9 years. The white cell count at the time of the experiment was

TABLE I

*Normal Blood — Average of Five Adults*

	Neutrophilic polymorphonuclears				Eosinophiles				Monocytes				Lymphocytes			
	1:16	1:64	1:256	C*	1:16	1:64	1:256	C	1:16	1:64	1:256	C	1:16	1:64	1:256	C
	100	98	36	12	83	52	7	4	91	90	41	14	0	0	0	0
Concentration of serum	1105	457	48	17	257	86	8	4	835	783	115	21	0	0	0	0
Percentage of cells containing bacteria																
Total number of bacteria ingested by 100 cells																

\* C = control (salt solution).

TABLE II

*Acute Lymphatic Leukemia*

	Oxidase-negative hemocytoblasts, lymphoblasts and promyelocytes				Lymphocytes				Neutrophils			
	1:16	1:64	1:256	C	1:16	1:64	1:256	C	1:16	1:64	1:256	C
	0	0	0	0	0	0	0	0	100	96	13	5
Concentration of serum									735	581	17	7
Percentage of cells containing bacteria												
Total number of bacteria ingested by 100 cells												



27,800, with myeloid hemocytoblasts or undifferentiated oxidase-positive cells 42 per cent, immature granulocytic cells (myeloblasts and promyelocytes) 16 per cent, and young granulocytes 18 per cent. The experiment showed that the hemocytoblasts and myeloblasts possessed but slight phagocytic activity; the metamyelocytes, rod nuclears and neutrophilic polymorphonuclears, or young granulocytic cells, showed a strong phagocytic activity, and that the lym-

TABLE III  
*Chronic Lymphatic Leukemia*

	Lymphocytic cells				Neutrophiles			
	1:16	1:64	1:256	C	1:16	1:64	1:256	C
Concentration of serum								
Percentage of cells containing bacteria	0	0	0	0	100	100	16	6
Total number of ingested bacteria	0	0	0	0	1223	983	19	8

phocytic cells showed no phagocytic activity whatsoever (Table IV). This experiment was repeated with similar results. However, in the second experiment the phagocytic power of the leukemic cells was tested, using both the patient's serum and pooled serum from normal adults; also leukocytes from healthy individuals were tested for phagocytic activity with both the patient's serum and the same mixed adult normal serum. The conclusion on this second group of experiments was as follows: first that the predominating leukemic cells (undifferentiated and immature oxidase-positive cells) showed slight or doubtful phagocytosis, either with the patient's serum or adult mixed serum; second, that the patient's young and mature granulocytes (over 5000 per cmm.) were actively phagocytic with both serums; and third, that the patient's serum had a high opsonic index. Nevertheless, the patient had severe mouth ulcerations. Türk cells showed no phagocytic activity.

*Chronic Myelogenous Leukemia:* Agnes M., a white female, aged 59 years, with a white cell count of 309,000, offered a classical picture of chronic myelogenous leukemia, both clinically and hematologically. The granulocytic cells were divided into six groups and the phagocytic activity appeared to be distributed as follows: myelo-

TABLE IV

*Acute Myelogenous Leukemia*

	Oxidase-positive hemocyto blasts and myeloblasts					Neutrophils				Lymphocytes			
	Promyelocytes		Myelocytes		C	Metamyelocytes		Rod nuclears		Polymorphonuclears		C	
	1:16	1:64	1:256	1:1024		1:16	1:64	1:256	1:1024	1:16	1:64	1:256	
Concentration of serum													
Percentage of cells containing bacteria	6	2	0	0	0	100	99	41	16	0	0	0	0
Total number of ingested bacteria	11	2	0	0	0	1500	1210	391	60	0	0	0	0

TABLE V

*Chronic Myelogenous Leukemia**Neutrophilic Cells*

	Myeloblasts				Promyelocytes				Myelocytes				Metamyelocytes				Rod nuclears				Polymorphonuclears			
	1:16		1:256		1:16		1:256		1:16		1:256		1:16		1:256		1:16		1:256		1:16		1:256	
	1:16	1:64	1:256	C	1:16	1:64	1:256	C	1:16	1:64	1:256	C	1:16	1:64	1:256	C	1:16	1:64	1:256	C	1:16	1:64	1:256	C
Concentration of serum																								
Percentage of cells containing bacteria	3	1	0	0	7	5	0	0	43	39	8	5	86	81	26	7	98	100	35	9	100	99	39	8
Total number of ingested bacteria	5	2	0	0	13	6	0	0	201	86	13	5	510	405	38	9	956	723	61	13	1015	810	70	14

blasts and hemocytoblasts, slight to doubtful; promyelocytes, slight; myelocytes, distinct phagocytosis; metamyelocytes, active phagocytosis; rod nuclears, active phagocytosis (more than the preceding group); polymorphonuclears, active phagocytosis (slightly more than preceding group). It appears, therefore, that all of the granulocytic cells show some degree of phagocytosis but that the appearance of maximal phagocytic activity is coincidental with the maturation of the cytoplasm and the appearance of the mature neutrophilic granulations (Table V).

Owing to the importance of these findings the experiment was repeated on another case of chronic myelogenous leukemia, Roy P., a white male, aged 29 years. The results fully confirmed the findings already stated. Basophilic cells show some phagocytic activity, but it appears to be much less than that of the neutrophils. Note that it is difficult to see bacteria in the basophilic cells.

*Acute Hemohistioblastic Leukemia:* Robert C., a negro, aged 35 years, presented an unusual case of extremely rapid hemohistioblastic or acute monoblastic leukemia. Diagnosis was made from the blood study and from biopsy of the sternum during life, confirmed at autopsy. The white cell count was 51,300 at the time of the experiment, with 83 per cent hemohistioblastic cells. Study was made on cells from the circulating blood as well as from an emulsion of cells from the sternum. The results showed that the hemohistioblastic cells have a strong phagocytic activity and that the monoblasts also have phagocytic activity but somewhat less than that of the hemohistioblastic cells (Table VI). The few polymorphonuclears present in this case also showed active phagocytosis. The finding that these highly undifferentiated cells behave in an entirely different fashion from the leukemic cells of other types is in full accord with the morphological implications. An emulsion of cells from the sternal marrow, obtained during life, showed that the hemohistioblastic cells predominated and that they were actively phagocytic toward a variety of particles, including bacteria, erythrocytes, carbon and collodion particles. This cellular emulsion was not washed. It contained an unknown quantity of the patient's serum and therefore results from this experiment are not strictly comparable. However, it appeared that collodion particles, large and small, and to a lesser degree carbon particles, were more actively phagocytosed by the hemohistioblastic cells than by neutrophilic polymorphonuclears.

TABLE VI  
*Acute Hemolistioblastic (Monoblastic) Leukemia*

	Hemolistioblasts				Monoblasts				Neutrophils			
	1:16		1:64		1:256		C		1:16		1:64	
	87		49		12		15		63		25	
Concentration of serum	515		408		13		15		290		128	
Percentage of cells containing bacteria												
Total number of ingested bacteria												

TABLE VII  
*Acute Hemolistioblastic (Monoblastic) Leukemia*

	Hemolistioblasts, monoblasts and monocytic cells				Immature granulocytic cells (myeloblasts and promyelocytes)				Mature granulocytic cells			
	1:16		1:64		1:256		C		1:16		1:64	
	61		48		21		23		7		2	
Concentration of serum	405		301		63		48		11		2	
Percentage of cells containing bacteria												
Total number of ingested bacteria												

These findings are particularly significant as they parallel differences in behavior of rabbits' exudative macrophages (monocytic cells) and polymorphonuclears toward small collodion particles.<sup>2</sup> Rieder cells of this same case appear to possess phagocytic power, approximately 30 per cent of them containing bacteria.

*Acute Hemohistioblastic Leukemia:* George H., a white male, aged 4½ years, showed a most unusual type of acute leukemia of the hemohistioblastic type (monoblastic) with a severe pneumonitis, probably terminal, and a large number of immature granulocytic cells. The white count at the time of the experiment was 131,000. Experiments again showed active phagocytosis by the hemohistioblastic, monoblastic and monocytic cells, slight phagocytosis by the immature granulocytic cells, including myeloblasts and promyelocytes, and the usual maximal phagocytic activity by the mature and young granulocytic cells including metamyelocytes, rod nuclears and polymorphonuclears (Table VII).

*Glandular Fever:* We also investigated the phagocytic activity of cells from a case of glandular fever (acute mononucleosis) which, although not connected with the leukemias, offers occasionally to the untrained some difficulty in the differential diagnosis. Joan G., a white female, aged 14 years, had at the peak of the attack a white cell count of 14,100; with the following differential count: rod nuclears 16, polymorphonuclears 41, eosinophiles 1, monocytes 3, prolymphocytes 1, lymphocytes 23, leukocytoid lymphocytes 3, and abnormal degenerated lymphocytic cells 12. It was particularly to test the phagocytic activity of this last group that the experiment on phagocytosis was performed. These cells are commonly classed as monocytes, although accurate morphological studies show them to be abnormal lymphocytes.<sup>3</sup> Strumia considers these cells degenerated forms of immature lymphocytic cells (lymphoblasts) but does not admit any relation to plasma cells. Phagocytosis experiments showed that these cells have no phagocytic activity whatever, giving further support to the view that these cells are lymphocytic.

#### BOERNER'S METHOD

This method is the same as that described by Boerner and Mudd,<sup>4</sup> and the results are summarized in Table VIII. One cc. of heparinized blood is placed in short test tubes (15 by 5 mm.) and placed in

TABLE VIII

Acute and Chronic Myelogenous Leukemia, Chronic Lymphatic Leukemia and Allergic Conditions

Case No.	Diagnosis	Organism	Serum	Hemocyto blasts	Myeloblasts	Promyelocytes	Myelocytes	Metamyelocytes and rod nuclears	Polymorphonuclear neutrophils	Eosinophiles	Lymphocytes	Monocytes
1	Acute myelogenous leukemia	Pneumo. 1 R " " Staph. albus	0 1:50 0	0 2 0					92 94 86			
2	Acute myelogenous leukemia	Staph. albus " " Pneumo. 1 R " "	0 1:10 0 1:50		1.5 1 4 2				100 100 99 99			
3	Chronic myelogenous leukemia	Pneumo. 1 S " 1 R " 1 R Staph. aureus " "	1:50 0 1:50 0 1:50		0 0 0 0 0	0 0 0.16 0 0	40 43 85 45 40	50 58 90 70 53	74 71 98 72 72			
4	Chronic myelogenous leukemia	Staph. aureus " " Pneumo. 1 R " "	0 1:50 0 1:50			0 2 6 4	50 53 64 57	50 62 90 90	65 70 88 94			
5	Allergic condition	Strep. Hem. (mucoid) Strep. Hem. (glossy) Pneumo. 1 S " " Yeast Erythrocytes	0 0 1:10 0 0 0						72 98 95 13 14 44	40 87 15 8 8 3		
6	Allergic condition	Pneumo. 1 S " 1 S Staph. aureus	0 1:75 0						26 100 100	4 64 100		
7	Chronic lymphatic leukemia	Staph. aureus " albus	0 0						100 100		0 0	94 86

Figures represent percentage of cells showing characteristic.

the agitator bath kept at a temperature of 37° C. In some instances 0.1 cc. homologous serum was added to the heparinized blood. To the blood or blood-serum mixture 0.1 cc. bacterial suspension in salt solution is added, containing 6 billion bacteria per cc., then after 15 minutes agitation smears are made and stained with Giemsa's solution. The antipneumococcus serum was obtained from the horse; the antistaphylococcic serum was of human origin. In counting, the percentage of cells showing ingested bacteria is noted.

CASE 1. Jeannette K., a white female, aged 17 years, suffering from acute myelogenous leukemia, showed at the time of experiment a leukocyte count of 12,000, with 75 per cent hemocytoblasts, the majority of which were oxidase-positive. The hemocytoblasts showed slight to doubtful phagocytic power; the mature polymorphonuclears active phagocytosis. In this experiment pneumococci and *Staph. albus* were employed.

CASE 2. Emma L., a white female, aged 60 years. Another case of acute myelogenous leukemia with a leukocyte count of 126,000 per cmm. at the time of experiment. The differential count showed a distinct prevalence of typical myeloblasts, forming with the more undifferentiated hemocytoblasts 82 per cent of the total circulating leukocytes. The myeloblasts showed a slight but definite phagocytic activity. The mature neutrophils showed an unusual maximal phagocytic activity. In this experiment pneumococci and *Staph. albus* were employed.

CASE 3. Mrs. Ethel C., a white female, aged 40 years, with chronic myelogenous leukemia, previously treated with X-ray. In this experiment the myeloblasts showed no phagocytic activity, the promyelocytes slight phagocytosis, the myelocytes, metamyelocytes and rod nuclears and the mature polymorphonuclears showed active phagocytosis, increasing slightly but definitely in each successive group in the order mentioned. In this experiment pneumococci and *Staph. aureus* were employed.

CASE 4. Roy P., a white male, aged 29 years, a case of long standing (7 years) chronic myelogenous leukemia, repeatedly treated with X-ray. About the time of experiment blood examinations showed a leukocyte count of over 100,000 per cmm., with about 80 per cent promyelocytes and all forms of granulocytic cells represented. Experiments employing *Staph. aureus* and pneumococci showed slight but definite phagocytosis with promyelocytes, active phagocytosis

for myelocytes, metamyelocytes and rod nuclears, and polymorphonuclears, with slight increase in the order given. Cells from Cases 3 and 4 showed closely similar behavior.

CASE 5. Horace L., a white male, aged 25 years, convalescing from asthma and colitis of allergic nature. This patient at the time of experiment had a total leukocyte count of 12,200, of which 28 per cent were neutrophils and 57 per cent eosinophils. This afforded a good opportunity for a comparative study of the two types of cells, with a variety of particles (two strains of hemolytic streptococcus, two of pneumococcus, yeast cells and erythrocytes). The eosinophils showed active phagocytosis, but always considerably below that of neutrophils, especially for certain type of particles.

CASE 6. Mary W. H., a white female, aged 44 years, presenting chronic follicular conjunctivitis showing on the day of experiment a leukocyte count of 8200 with 55 per cent neutrophils and 19 per cent eosinophils. With pneumococci phagocytosis appeared definitely less with the eosinophils than with the neutrophils. With a strain of *Staph. aureus* the percentage of phagocytosis was maximal with both types of cells, but the total number of bacteria ingested by the eosinophils was far below that ingested by the same number of neutrophils (see Table IX). This is also apparent from Table I giving data from normal blood.

CASE 7. Harry D., an adult white male, suffering from chronic lymphatic leukemia treated with X-ray. Prior to experiment blood examinations had shown a leukocytic content of over 200,000 per cmm., with a predominance of young lymphocytic cells (prolymphocytes). Experiment showed a maximal phagocytosis with the neutrophils, only slightly less active phagocytosis with monocytes, and no phagocytosis with any of the lymphocytic cells. *Staph. aureus* and *Staph. albus* were used in this experiment.

## DISCUSSION

Making due allowance for the great variation in the state of preservation of leukemic cells, for the difference in the methods used in the two series, and for the difference in the particles employed, the results are remarkably uniform. In the first series, using Strumia's method, the washing of the cells has the great advantage of eliminating the variations of the opsonic value of the patient's serum,



TABLE IX

Case 6

*Pneumococcus Type 1 S*

	No. bacteria per cell	Time in minutes				
		3	6	9	12	15
Eosinophiles	over 10	0	0	0	0	0
	5 to 10	0	0	0	0	0
	1 to 5	1	6	3	8	4
Neutrophiles	over 10	1	1	0	1	0
	5 to 10	2	1	0	0	1
	1 to 5	18	13	8	12	26

*Pneumococcus Type 1 S**Antipneumococcus serum (1:75)*

Eosinophiles	over 10	6	8	11	17	10
	5 to 10	10	12	11	16	18
	1 to 5	28	35	29	31	36
Neutrophiles	over 10	30	65	90	93	88
	5 to 10	20	19	5	5	4
	1 to 5	20	15	3	2	8

*Staphylococcus aureus*

Eosinophiles	over 10	23	68	68	72	80
	5 to 10	36	16	15	13	10
	1 to 5	35	12	13	12	10
Neutrophiles	over 10	66	100	99	100	100
	5 to 10	28	0	0	0	0
	1 to 5	6	0	1	0	0

substituting progressive dilution of a serum pooled from ten or more patients, which has proved to be remarkably constant in its opsonic value, at least with the strain of staphylococcus used, thus affording a comparable series of cases. The counting of the actual number of bacteria phagocytosed in addition to the percentage of cells showing phagocytosis, affords a much better quantitative measure of the phenomenon. A disadvantage of the method is the greater time required and the greater chance of cell damage. Both of these factors can be reduced to a minimum by carefully following the technic.

The advantages of the method of Boerner and Mudd are its simplicity of technic, the small amount of blood required, and above all that the leukocytes have undergone only a minimum of manipulation and are functioning in their natural medium. In later work with this method the number of bacteria per cell, as well as the percentage of phagocytizing leukocytes has been counted. The disadvantage of the method is the undetermined variation of the opsonic index of the patient's serum, which renders the data less reliable for comparative study.

Although leukemic cells are probably not identical in function with normal cells, they are similar enough so that conclusions concerning their phagocytic activity may be expected roughly to apply to normal cells of corresponding development. This statement is based on comparative studies made by one of us (Strumia) although not enough experimental data are on hand to justify final conclusions. As the work stands, the phagocytic activity of mature polymorphonuclears and eosinophiles from fresh human rib marrow emulsion is comparable to that of the same cells from circulating blood, although usually less. Lymphocytic cells from lymph nodes of rabbits, and lymph nodes removed at autopsy from children within 6 hours of death, show no phagocytosis. Monocytic cells from a splenic emulsion (from an accident case within 3 hours of death) freed as much as possible of blood, show active phagocytosis. Many of these cells, however, appear to be younger than the circulating monocytes (monoblasts and hemohistioblasts).

However, when we wish to study young and immature cells from a non-leukemic subject the variations are greater. Thus, immature and young granulocytic cells from fresh human rib marrow have usually less phagocytic activity than the corresponding leukemic circulating cells; whereas circulating young granulocytic cells,

metamyelocytes and rod nuclears, from cases of infections with mild toxemia, show generally more phagocytosis than the corresponding leukemic circulating cells.

It is essential to avoid cell injury as much as possible and, in studying circulating leukocytes in cases of infections, to use only patients whose blood shows on ordinary stained smears no signs of severe degenerative changes of the leukocytes.

### SUMMARY AND CONCLUSIONS

Lymphocytic cells never show phagocytosis. Undifferentiated oxidase-negative cells (lymphoid hemocytoblasts) likewise show no phagocytic activity. Granulocytic cells and their precursors constantly show active phagocytosis, as follows: undifferentiated oxidase-positive cells (myeloid hemocytoblasts) show slight phagocytic activity in some experiments, doubtful in others. The immature cells (myeloblasts and promyelocytes) show a slight but definite and progressive increase in the phagocytic activity. It is to be noted that the promyelocyte, which is the first cell of the granulocytic series to show the appearance of the specific neutrophilic (or eosinophilic) granulation, is also the first cell showing a definite phagocytic activity in all experiments conducted.

The myelocyte, the first cell to possess an entirely mature cytoplasm, namely an acidophilic cytoplasm, with mature (neutrophilic) granulations, shows phagocytic activity greatly increased over the preceding cells. There is further increase in the phagocytic activity with the other granulocytic type of cells, namely metamyelocytes, rod nuclears and polymorphonuclears. The possible relation of phagocytosis to the area of cytoplasm was considered and investigated. The phagocytic activity of the myelocyte, metamyelocyte, rod nuclear and mature polymorphonuclear obtained from a number of experiments on leukemic and non-leukemic blood, compared with the relative area of the cytoplasm in smears of these cells, can be summarized as follows:

Neutrophilic cells	Phagocytic activity*	Area of cytoplasm exclusive of nucleus†
Myelocytes	30	86
Metamyelocytes	67	90
Rod nuclears	96	97
Polymorphonuclears	100	100

\* Per cent of cells showing phagocytosis.

† Relative surface of cytoplasm on stained preparations, taking that of the neutrophilic polymorphonuclears as equaling 100.

In the above table, while for the myelocyte and the metamyelocyte there is no parallelism between the surface of the cell cytoplasm and its phagocytic activity, a parallelism between the two figures exists in the rod nuclear and the polymorphonuclear. It is interesting to note that the rod nuclear only of the young cells is normally present in circulation. One may conclude that in so far as the phagocytic activity is concerned there is no difference between the mature polymorphonuclear and the rod nuclear.

Eosinophiles show phagocytic activity but far less than either the neutrophiles or the monocytes. It is to be noted that while a fair percentage of eosinophiles shows phagocytosis, each cell usually contains fewer bacteria. This can readily be seen from Table I (normal blood), and from Table VIII. The basophiles show even less phagocytic activity, but allowance must be made for the fact that it is difficult to differentiate between granules and bacteria.

Monocytic cells (hemohistioblasts, monoblasts, monocytes) constantly show active phagocytosis. In so far as the bacteria employed are concerned monocytes appear somewhat less active than neutrophilic polymorphonuclears, but collodion granules and carbon particles appear to be picked up more readily by monocytic cells. Rieder's cells show slight but definite phagocytic activity; Türck's cells show none.

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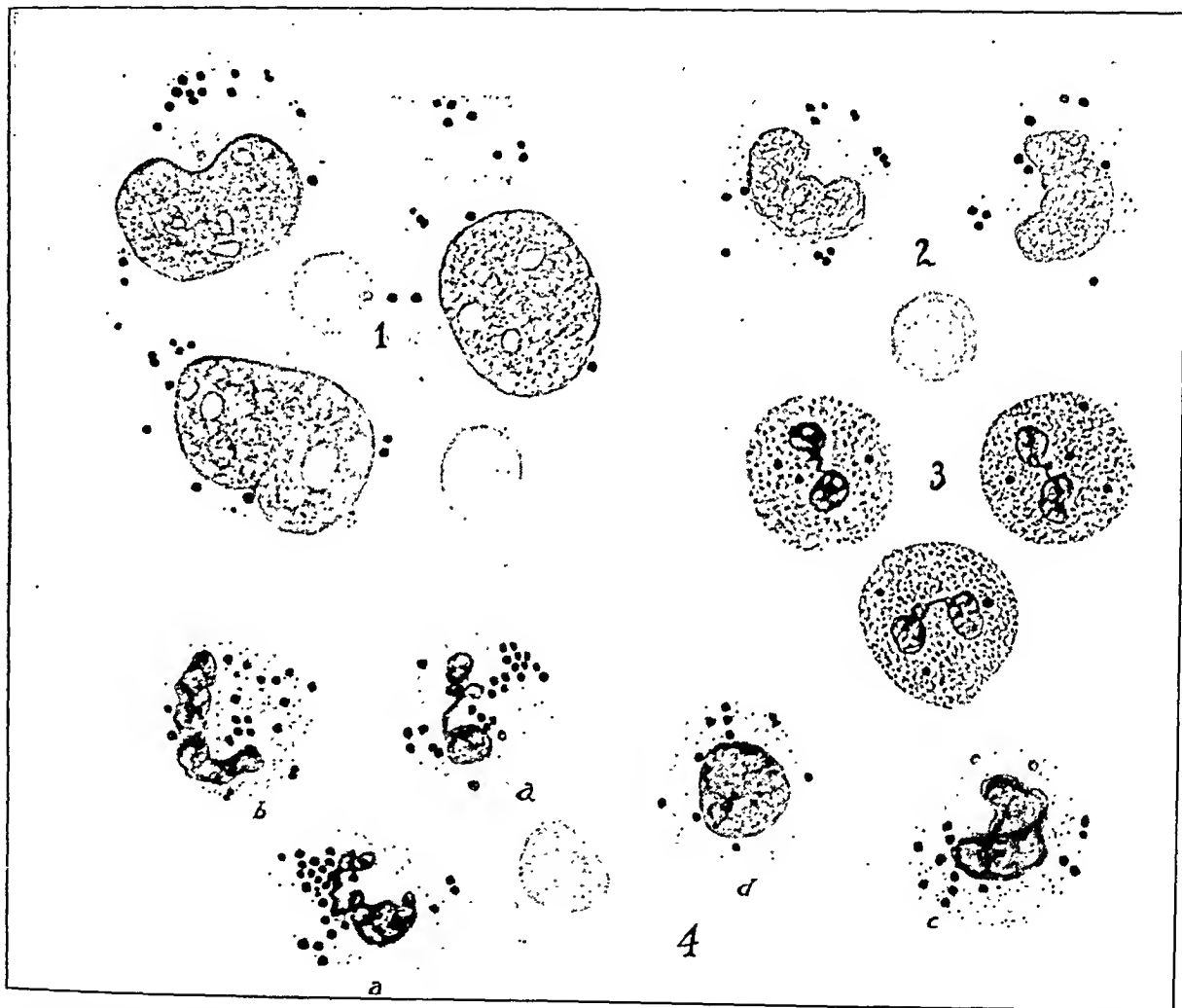
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## DESCRIPTION OF PLATE

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### PLATE 57

- FIG. 1. Hemohistioblasts from a case of acute hemohistioblastic (monoblastic) leukemia showing phagocytosis of staphylococci (Strumia's method).
- FIG. 2. Monocytes from normal circulating blood with phagocytozed staphylococci.
- FIG. 3. Eosinophiles from normal circulating blood with phagocytozed staphylococci.
- FIG. 4. Neutrophilic cells from a case of chronic myelogenous leukemia showing phagocytozed staphylococci. a = mature polymorphonuclears; b = rod nuclear; c = metamyelocyte; and d = myelocyte.



Strumia and Boerner

Phagocytic Activity of Circulating Cells



## THE PANETH CELL \*

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In the small intestine of man and many animals there exists at the base of the crypts of Lieberkühn a striking eosinophilic cell. Schwalbe,<sup>1</sup> in 1872, was the first to describe these cells. It remained for Paneth,<sup>2</sup> in 1888, to bring them to the attention of the investigators and for this reason they are known as Paneth cells. Paneth described them at some length and as a result of his efforts they became recognized as constant constituents of the intestinal glands of certain animals. Paneth regarded these granular cells as a specific kind of gland cell wholly different from the goblet cell. In 1892 Bizzozero<sup>3</sup> claimed to have found in material from the intestine of the mouse transitional forms between the Paneth cell and the goblet cell. He assumed, therefore, that the Paneth cell was a young goblet cell. Möller,<sup>4</sup> in 1899, found Paneth cells in the intestinal glands of the guinea pig, rabbit, ox, sheep and horse. He was unable to demonstrate them in the pig, cat and dog. His observations led him to conclude that the Paneth cell possessed a true secretory function. In 1906 Klein<sup>5</sup> found basal filaments in the cytoplasm of Paneth cells of the guinea pig. He found that the cells responded to physiological stimulation, which led him to consider that they represent a zymogenic cell involved in digestion. In 1905 Schmidt<sup>6</sup> studied the distribution of the cells of Paneth in man and confirmed Bloch's<sup>7</sup> observation (1903) that they occurred in practically every gland of the ileum and jejunum as well as the duodenum. In addition, Schmidt found them present frequently in the glands of the appendix, although he was not able to find them in other portions of the large intestine, except in 3 cases in which pathological conditions were present. Concerning their occurrence in the large intestine of infants, where Bloch claims to have observed them, Schmidt recorded a negative result in five newborn infants. For the differentiation between goblet cells and Paneth cells he used mucicarmine. Prenant,<sup>8</sup> in 1907, because of a similarity in staining of the goblet cell and the

\* This work was started while the author was a Fellow in Pathology, The Mayo Foundation, Rochester, Minnesota.

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Paneth cell with a "vert-lumière" stain, concluded that the Paneth cells were mucus cells but that they represented specific types that were independent of the goblet cells. Bensley,<sup>9</sup> in 1908, held that Prenant's "vert-lumière" stain was not a specific stain for mucus, and in view of his observations concluded that the Paneth cell resembles a zymogenic rather than a mucus cell. Stöhr and Lewis,<sup>10</sup> in 1913, reported Paneth cells as occurring in the duodenum, jejunum and ileum of man but not in the large intestine. They said that the Paneth cell probably produces a special secretion which enters the lumen of the gland in the form of fine granules when the digestion of fat is taking place and that it may perhaps also be concerned with protein, but not with carbohydrate digestion. Chuma,<sup>11</sup> in 1923, in a study of the gastric mucosa in both normal and pathological conditions, mentioned the occasional occurrence of Paneth cells in pathological gastric conditions. Clara,<sup>12</sup> in 1926, concluded that the Paneth cell, although it did not normally arise from the principal epithelial cells of the mucosa in man, might originate from these cells under certain conditions, since it is known that in cases of chronic intestinal irritation, such as produced by carcinoma, the number of Paneth cells might be increased. He also reported their occurrence in the large intestine of domestic animals. Mols,<sup>13</sup> in 1930, studied the effects of different diets on the Paneth cells of mice. He concluded that the Paneth cells are a type of serous cell. Baecker,<sup>14</sup> in 1934, wrote an extensive article on Paneth cells and summed up their occurrence in various forms of animal life. He expressed the opinion that these cells were widely distributed in ancestral forms of life and gradually became localized in the crypts. He also said that the Paneth cell and goblet cell are closely related but that the Paneth cell, because of its wide distribution in nature, constitutes a definite cell type. He was unable to find in lower animal life a predecessor for both the Paneth cell and the goblet cell. Cowdry<sup>15</sup> considered the possibility that the Paneth cells and the enterochromaffin cells might be the source of the duodenal hormones, secretin and cholecystokinin. However, he concluded that this is highly improbable as they are found in regions of the intestine that do not manufacture hormones; also, the Paneth cell looks like an exocrin cell discharging its granules directly into the lumen of the crypts. He concluded by saying that what they produce is a mystery as the reports following the use of different diets are conflicting.

Boerner-Patzelt,<sup>16</sup> in 1935, studied the influence of changing the pH of the fixative on the staining characteristics of the granules of the Paneth cells. By regulating the pH of a concentrated aqueous solution of picric acid and using a stain composed of methylene blue and kresponceau, some of the granules in the same cell took the blue stain while others took the red stain. She thought this variation in staining depended on the age of the secretion granules. She concluded that since the isoelectric points of the Paneth cell and goblet cell do have the same pH, a close relation between the two cells does not seem improbable.

In view of the many conflicting reports concerning these cells and the fact that the majority of the work done has concerned their occurrence in lower animal life, an investigation under normal and pathological conditions in human beings seemed in order.

### MATERIAL

The greater part of the material used for this study was obtained at fifty routine autopsies. The sections from the intestine were secured as soon as possible at the autopsy table and placed in Orth's fixing fluid. The routine stain used was hematoxylin and eosin. In addition, special fixatives and stains were used, which will be described later. In order to study fresh material several sections of small intestine were secured from the operating room. Because of the difficulty in securing fresh human material, additional material was secured from guinea pigs that had been fasting for 12 hours. The time that elapsed between death and autopsy varied from 1 to 7 hours. In cases in which more than 7 hours had elapsed between death and autopsy the material usually was not suitable for routine study, and in many cases in which less than 7 hours had elapsed following death the material had to be excluded because postmortem changes made cytological studies impossible.

### MORPHOLOGY

The Paneth cell is a flask shaped cell with a broad base in contact with the basement membrane of the crypt of Lieberkühn and a narrow apex that reaches the lumen of the crypt. The cell possesses a round spheroidal nucleus, which is poor in chromatin and is situated near the base of the cell. Usually a distinct nucleolus is seen. The cytoplasm is light staining and the distal portion above

the nucleus is filled with large, round, brilliant eosinophilic granules which are all approximately the same size (Fig. 1). It is interesting to note that no granules were ever seen outside of the cell within the lumen of the crypts.

#### SITUATION AND DISTRIBUTION UNDER NORMAL AND PATHOLOGICAL CONDITIONS

In the small intestine of man the Paneth cells are seen at the very base of the crypts of Lieberkühn. They fill the distal ends of the crypts. When the lateral walls of the base of the crypts are examined goblet cells are found lying in apposition to the Paneth cells. The incidence of goblet cells then increases as the villi are approached. Kaufmann-Wolf,<sup>17</sup> in 1911, reported the occasional occurrence of Paneth cells in the villi of the small intestine, but I was not able to confirm this finding. In this series of 50 cases Paneth cells were found within the crypts of every section of the small intestine examined, except that of a 4½ months intrauterine fetus, which contained goblet cells but no Paneth cells.

—In order to study the normal incidence of Paneth cells among the crypts of the different portions of the small intestine of man a count was made with the material obtained from these 50 cases. This was done by counting twenty crypts in sections obtained from the duodenum, jejunum and ileum of each of the 50 cases. The count was made in the field that represented the most typical structure of the section. Out of the twenty crypts counted the number containing Paneth cells was noted. Hence, if ten of the twenty crypts contained Paneth cells the incidence in that section would be 50 per cent. The incidence in the different portions of the small intestine was as follows: in the duodenum, 48.5 per cent; in the jejunum, 74 per cent; and in the ileum, 73.5 per cent. The Paneth cells, therefore, seem to be most numerous in the jejunum and ileum, where their number is practically equal. They are found here in approximately three of every four crypts. In the duodenum their number is definitely less and they are found in approximately two of every four crypts.

Paneth cells do not normally occur in the stomach. I am indebted to Dr. Charles Baker,<sup>18</sup> who recently made an interesting morphological study of the gastric mucosa and whose material I had an opportunity to examine. Paneth cells were not found in the normal stomach. In 50 per cent of a series of 100 cases of ulcer and

carcinoma of the stomach, abnormal intestinal-like mucosa was found in some regions. When this type of mucosa existed Paneth cells were found at the bottom of the crypts in 98 per cent of the cases (Fig. 2).

Paneth cells occasionally occur in the large intestine, including the appendix. The appendix and colon offered an opportunity to study further the effect of abnormalities of the mucosa, as found in inflammation, malignant conditions and granulomatous lesions, on the occurrence of Paneth cells.

In a study of the appendix Paneth cells were present in two of twenty-five apparently normal appendixes. Paneth cells were present in three of twenty-five diseased appendixes. Hence, inflammation seems to have little effect on the presence of Paneth cells in the appendix. Moreover, when they were present in the appendix their incidence was much less than it was in the small intestine.

The 50 cases in which material was obtained from the cecum, colon and rectum were divided into two groups. The first group includes 25 cases in which the large intestine appeared to be normal. Paneth cells were not found in the large intestine in this group of cases. The second group consists of 25 cases in which the large intestine was the site of a malignant growth, tuberculosis or chronic ulcerative colitis. Paneth cells were found in 3 cases in the latter group; 2 of these cases were tuberculosis of the cecum, and the 3rd was a case of chronic ulcerative colitis. In the cases in which Paneth cells were present their incidence was similar to that in the appendix, that is, it was less than the normal occurrence as established for the normal small intestine.

It is interesting to note that when Paneth cells exist in the stomach or colon in association with any malignant condition, usually the growth is strongly eosinophilic, staining intensely with eosin at the expense of the basophilic structures. There is also much condensed mucus present.

The subjects of this study ranged from a  $4\frac{1}{2}$  months intrauterine fetus to an adult of 80 years. Age did not seem to have any influence on the occurrence of Paneth cells except in the  $4\frac{1}{2}$  months fetus, in which case goblet cells but no Paneth cells could be demonstrated in the small intestine. Specimens were obtained from two infants who were born prematurely at the 7th and 8th months of pregnancy respectively. In both of these cases autopsy, which was performed

soon after death, revealed that Paneth cells and goblet cells were present in normal quantities. Sex did not seem to have any influence on the occurrence of these cells.

Paneth cells do not occur normally outside of the gastro-intestinal tract, but a Paneth cell was found in the pancreatic duct near its distal end. In the guinea pigs studied the Paneth cells showed little difference, other than in size, from those that occur in human beings.

### FIXATIVES

In order to determine the effect of different fixatives on the Paneth granules separate sections of the small intestine of a guinea pig were placed in Bouin's, Orth's, Zenker's, Helly's and Bensley's fixatives, in absolute alcohol, and in a 10 per cent solution of formalin. They were then stained with hematoxylin and eosin. The effects of these fixatives on the Paneth granules varied only slightly. One, therefore, can conclude that the ordinary fixatives preserve the granules and that the choice of a fixative is determined largely by the staining method to be used. However, the best results as far as the distinctiveness and brilliance of the granules were concerned were obtained with Bouin's fixative.

### STAINING

The Paneth granules stain well with acid dyes such as eosin and acid rubin. Various differential stains have been used to study these cells. Mucicarmine fails to stain the Paneth granules but, as would be expected, it stains the mucus within the goblet cells a brilliant red color. The Paneth granules are not stained by silver salts. An attempt was made to demonstrate a staining similarity between these cells and such substances as corpora amylacea of the prostate gland, lungs and central nervous system, and chondroproteins such as amyloid and similar substances. The results were pleasing. Congo red, a stain commonly employed in the study of amyloid material, brings out the Paneth granules distinctly, much more so than does eosin (Fig. 1). At times it is extremely difficult to say whether a section contains Paneth granules or not, with the use of hematoxylin and eosin, especially when the section is heavily stained with eosin. Congo red is particularly useful in these cases.

Paneth granules are stained a brilliant green color by de Galan-

tha's<sup>19</sup> amyloid stain, which contains carbol fuchsin, orange S, indigo carmine and picric acid. In an attempt to find Bizzozero's<sup>20</sup> transitional forms of Paneth cells, which contain both Paneth granules and mucus, it was noticed that the sections stained with ordinary hematoxylin and eosin did not have sufficient color contrast. Hence, a method was sought that would stain the Paneth granules one color, and at the same time stain the mucus within the goblet cells another. De Galantha's amyloid stain, when combined with mucicarmine, was found very satisfactory as the Paneth granules were stained a bright green and the mucus within the goblet cells a brilliant red. On careful examination of the crypts of Lieberkühn stained by this method it was found that one or two cells of a section situated at the base of the crypts contained numerous Paneth granules stained green and, in addition, red-staining droplets of mucus were present near the distal end of the cell. This finding was so striking that twenty sections of human small intestine stained with this technic were studied. The Paneth cells and goblet cells seemed to be independent of each other, but diligent search would show that from one to three cells of the section, which were situated at the base of the crypts, contained both the green granules and the mucus droplets. However, this was not found in every case studied as 7 of the 20 cases examined failed to reveal this phenomenon.

Best's carmine stain was first tried on human material that had been secured at autopsy and fixed in absolute alcohol. The results were negative. However, when the procedure was repeated on fresh specimens obtained from guinea pigs the Paneth granules gave a positive reaction for the presence of glycogen. Meriwether<sup>21</sup> has shown that corpora amylacea, both in the prostate gland and in the central nervous system, when stained with this stain, are found to contain glycogen.

#### COMMENT

The question is, what is the nature of the Paneth cells? The literature is divided into two schools: one considers the Paneth cell an independent zymogenic cell that possesses some unknown function; the other thinks it is closely related to the mucus cells of the intestine. Everything else being equal, it is easier to think of the Paneth cell as probably representing a cell closely related to the mucoid goblet cell rather than as an independent zymogenic cell.

Definite staining similarities exist between the Paneth granules and substances such as corpora amylacea of the prostate gland and amyloid material. The presence of Paneth cells out of their normal habitat, associated with pathological conditions as in carcinoma of the stomach, is extremely significant. When these cells are found associated with a carcinoma of the stomach there is usually a large quantity of coagulated mucus present. This would indicate to me that the Paneth cells are closely related to the production of mucus. Finally, de Galantha's stain demonstrates an occasional Paneth cell that seems to contain droplets of mucus as well as Paneth granules. However, this is not constant but could be demonstrated in a few crypts in 13 out of 20 cases observed. Bizzozzero claimed to have found the same phenomenon and advocated the theory that the crypts of Lieberkühn acted as regenerative foci and that the Paneth cell is an early form of mucus cell. Prenant regarded these cells as mucus cells but of a different type from the goblet cell, as the Paneth cells are not stained by mucicarmine. If the Paneth cells are mucoid in nature their exact relation to the goblet cell is difficult to state, as today investigators are still somewhat uncertain as to the origin of the mucoid goblet cell. The Paneth cell could be regarded as a mucoid cell independent of the goblet cell, or as a primitive type of goblet cell. However, the exact nature of the Paneth cell will probably remain unknown until more definite microchemical reactions are utilized.

### CONCLUSIONS

1. Paneth cells are coarsely granular cells found constantly in the small intestine of man.
2. They are most numerous in the jejunum and ileum, where their number is practically equal. They occur less frequently in the duodenum.
3. Paneth cells may occur occasionally in the colon and appendix, and also in the stomach, under pathological conditions.
4. Age and sex do not influence their occurrence.
5. The granules are easily preserved by the ordinary fixatives.
6. The Paneth cell gives evidence of being mucoid in character rather than an independent zymogenic cell.

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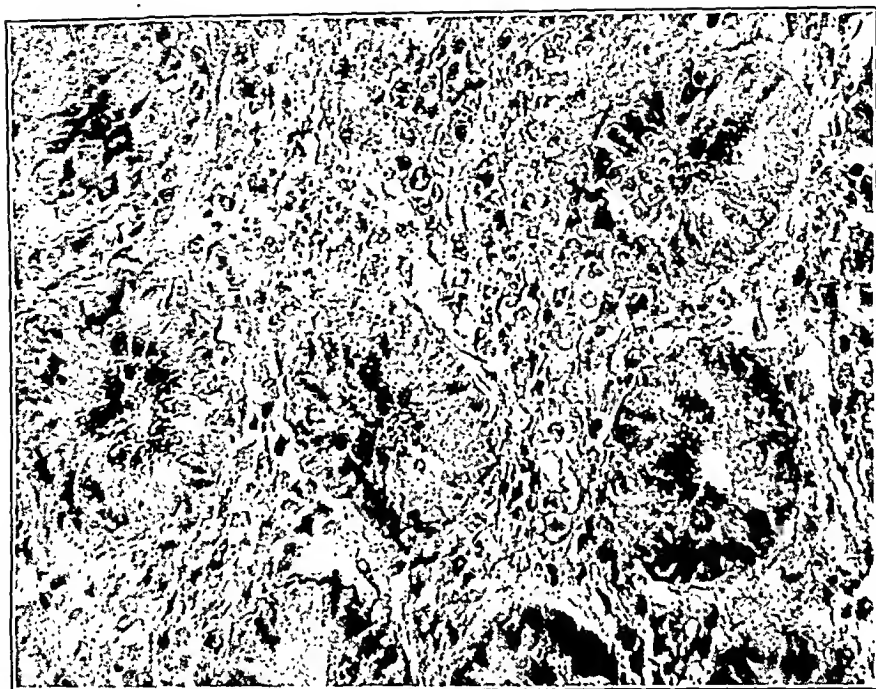
### DESCRIPTION OF PLATE

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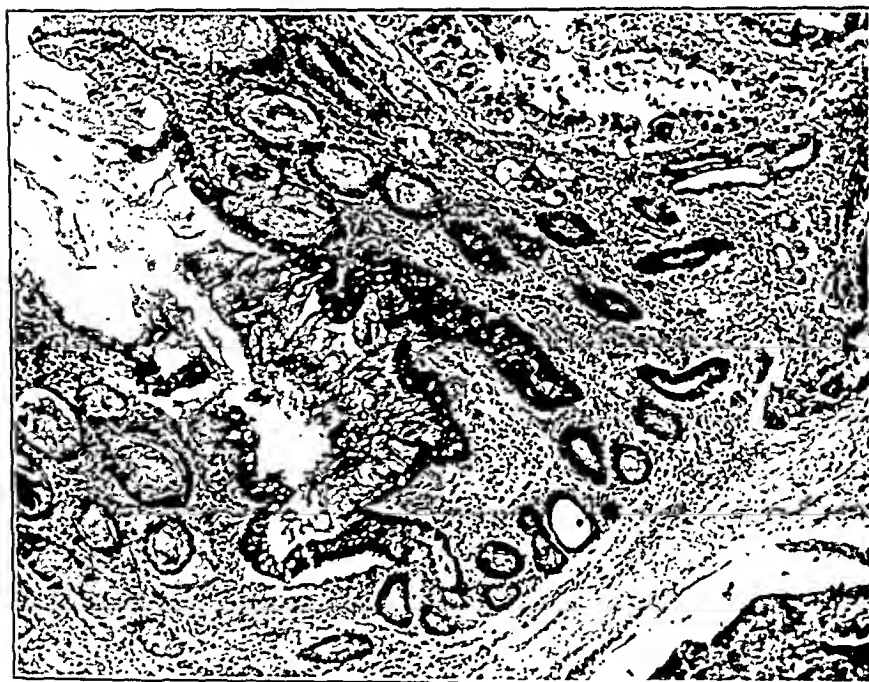
#### PLATE 58

FIG. 1. Human ileum. Bouin's fixation, Congo red stain.  $\times 415$ .

FIG. 2. Carcinoma of human stomach. The mucosa adjacent to the malignant cells is of the intestinal type; green-staining Paneth granules are present in these crypts. A large amount of mucus is associated. Orth's fixation, de Galantha's orange S plus Mayer's mucicarmin stain.  $\times 70$ .



I



2

Hertzog

The Paneth Cell



# CULTIVATION OF THE VIRUS OF ST. LOUIS ENCEPHALITIS \*

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Among the various methods of tissue culture that have been adapted to the cultivation of viruses *in vitro* the techniques devised by Maitland and Maitland <sup>1</sup> and by Li and Rivers <sup>2</sup> have been widely employed for this purpose. Using similar conditions of cultivation Syverton and Berry <sup>3</sup> have recently demonstrated growth of the virus of St. Louis encephalitis <sup>4, 5</sup> in a medium composed of minced mouse embryo tissue, rabbit serum and Tyrode's solution.

The experiments to be reported in the present paper confirm the observations of Syverton and Berry and, in addition, reveal that the virus may be propagated in media containing chick embryo tissue, as well as in developing eggs, by the method of Woodruff and Goodpasture. <sup>6</sup>

## MATERIALS AND METHODS

### *Strains of Virus*

Three strains of the virus employed by one of us (E. M.) in previous experiments <sup>7</sup> were used in the present study; two (Daily and Barnes) were originally isolated by Muckenfuss, Armstrong and McCordock, <sup>4</sup> and the other (No. 3) by Webster and Fite. <sup>5</sup> Infected mouse brain, frozen and dried 7 months previously, was inoculated intracerebrally into mice, and the supernatant fluid of a 10 per cent brain emulsion from the 3rd mouse passage was used to initiate the cultures.

### *Methods of Cultivation*

In preliminary experiments on cultivation of the virus a study was made of *in vitro* and *in vivo* methods. The media chosen for use were: A, that described by Syverton and Berry, <sup>3</sup> composed of finely minced living mouse embryo tissue suspended in fluid consisting of 1.7 cc. of Tyrode's solution and 1 cc. of fresh normal rabbit serum, and distributed in 50 cc. Erlenmeyer flasks; B, similar medium con-

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taining 2.7 cc. of Tyrode's solution without serum; C, minced chick embryo tissue, Tyrode's solution and rabbit serum in the same proportions as in medium A; and D, chick embryo tissue in 2.7 cc. of Tyrode's solution with no serum. The tissue inoculum of each flask was approximately 0.02 gm. of finely minced tissue from mouse embryos removed during the final week ante partum and from chick embryos incubated for 9 or 10 days. After inoculation with 0.3 cc. of virus suspension the flasks were stoppered with cotton, sealed with tinfoil and incubated at 37.5° C. for 4 or 5 days. Inoculations were made in quadruplicate in each medium. Cultures were examined for bacterial contamination routinely by direct smear or subculture.

For studying cultivation of the virus in the presence of antiviral serum media were prepared as in A except that immune serum was substituted for normal rabbit serum. The difficulty of obtaining convalescent serum of sufficiently high neutralizing power for the encephalitis virus necessitated the preparation of immune serums in animals. This was accomplished by injecting rabbits with the Daily strain intramuscularly three times at weekly intervals with 1 cc. of supernatant fluid from freshly prepared 10 per cent suspensions of brains of mice dying from encephalitis. Protection tests were performed, as previously described,<sup>7</sup> to determine the neutralizing capacity of the rabbit immune serums. Mice inoculated intracerebrally with 0.25 cc. of serum-virus mixtures containing  $10^{-3}$ ,  $10^{-4}$  and  $10^{-5}$  dilutions of virus were protected. Under these conditions of immunization each of four rabbits responded with serums of this titer.

In addition to growth by *in vitro* methods the virus was cultivated in the fertilized hen's egg according to the method of Woodruff and Goodpasture. In later experiments artificial air sacs were produced by the technique of Burnet and Galloway.<sup>8</sup> Inoculations were made by placing two drops of virus suspension on the chorio-allantoic membrane. Undiluted flask culture material was used for the initial inoculum and subsequently 10 per cent suspensions of infected chorio-allantoic membranes served for serial passage of the virus. Usually six or more eggs were inoculated for each passage. Eggs were incubated at 38° C. and were observed twice daily. They were opened for final examination on the 2nd to the 10th day after inoculation.

*Demonstration of Virus in Culture*

The presence of virus in culture media was determined by intracerebral inoculation of mice under ether anesthesia. Tissue, suspended in fluid from the same culture flasks, was ground aseptically without abrasive. After centrifugation for 5 minutes at 2500 r.p.m. the supernatant fluid was injected undiluted or, when titrations were made, diluted with Tyrode's solution to the proper concentration. The C-57 strain of black mice was used except in rare instances when white mice from local stocks were injected. To determine the presence of virus in the inoculated eggs the supernatant fluid from a 10 per cent suspension of ground chorio-allantoic membrane was employed. In testing for virus in a given culture or egg passage generally three, occasionally four or more, mice were inoculated.

The clinical and pathological changes observed in mice infected with virus cultivated in eggs and in the various types of media were identical with those following injection of mouse passage virus.<sup>9</sup>

## EXPERIMENTAL

*Cultivation in Vitro*

As may be seen from the data recorded in Table I, three strains of the virus of St. Louis encephalitis have been grown in each of the four media described. In medium A, containing minced mouse embryo tissue, normal rabbit serum and Tyrode's solution, the Daily strain of virus was cultivated through 26 transplants over a period of 4 months, while the Barnes strain was carried through 25 transplants and the Webster No. 3 strain through 8.

Since growth of the virus was not recognizable visibly in any of the media it was necessary to determine the presence of virus by injecting the cultures into susceptible animals. Equal quantities of material from the quadruplicate culture flasks were pooled and injected undiluted into three or more mice for routine testing. The infectivity of successive generations of subcultures was determined at frequent intervals. Culture material from all three strains was infectious for mice when the experiments were discontinued.

In B, mouse embryo medium containing no serum, the cultivation of virus was less successful. The Webster No. 3 strain failed to survive the first few transplants on three different occasions, and the Barnes strain was lost between the 11th and 14th serial transplants.

TABLE I

*Cultivation of St. Louis Encephalitis Virus in Vitro*

Tissue cultures		Source of virus	Culture generations in source medium	Cultures in test medium			Total generations in culture	Calculated dilution of original virus in last virulent culture	
Medium	Virus strain			Number of generations	Generations fatal for mice*	Generations not fatal for mice		Since inoculated in test medium	Since isolation from mice
A Mouse embryo in rabbit serum and Tyrode's solution	Barnes	Mouse brain	—	25	6th, 10th, 12th, 15th, 17th, 18th, 19th, 21st, 25th		25	10 <sup>-26</sup>	10 <sup>-26</sup>
	Daily	Mouse brain	—	26	6th, 12th, 15th, 17th, 18th, 20th, 22nd, 23rd, 24th, 26th		26	10 <sup>-27</sup>	10 <sup>-27</sup>
	Webster	Mouse brain	—	8	1st, 3rd, 5th, 8th		8	10 <sup>-9</sup>	10 <sup>-9</sup>
B Mouse embryo in Tyrode's solution	Barnes	Culture in medium A	10	14	2nd, 5th, 7th, 8th, 9th, 11th	14th	24	10 <sup>-12</sup>	10 <sup>-22</sup>
	Daily	Culture in medium A	9	15	5th, 7th, 8th, 12th, 15th	10th	24	10 <sup>-16</sup>	10 <sup>-25</sup>
		Culture in medium A	5	5		5th	10		
	Webster	Mouse brain	—	5		1st, 3rd, 5th	5		
C Chick embryo in rabbit serum and Tyrode's solution		Culture in medium A	6	3	1st	3rd	9	10 <sup>-2</sup>	10 <sup>-8</sup>
		Culture in medium D	5	4	2nd, 4th		9	10 <sup>-5</sup>	10 <sup>-10</sup>
	Barnes	Culture in medium A	4	14	6th, 11th	5th, 13th, 14th	18	10 <sup>-12</sup>	10 <sup>-16</sup>
	Daily	Culture in medium A	4	20	5th, 11th, 13th, 14th, 16th, 18th, 20th		24	10 <sup>-21</sup>	10 <sup>-25</sup>
	Webster	Mouse brain	—	8	1st, 3rd, 5th, 8th		8	10 <sup>-9</sup>	10 <sup>-9</sup>
	Barnes	Culture in medium C	11	9	3rd, 7th, 9th†		20	10 <sup>-10</sup>	10 <sup>-21</sup>
D Chick embryo in Tyrode's solution	Daily	Culture in medium C	11	9	3rd, 5th, 7th, 9th		20	10 <sup>-10</sup>	10 <sup>-21</sup>
	Webster	Mouse brain	—	8	1st, 3rd, 5th, 8th		8	10 <sup>-9</sup>	10 <sup>-9</sup>

\* Three mice were usually inoculated, occasionally four or more, with each culture. Except where noted all mice died in from 4 to 7 days with typical convulsions.

† One mouse escaped, one survived, one died with convulsions.

However, the Daily strain was still infectious for mice in the 15th generation when the experiment was discontinued.

Cultures of the Daily strain were maintained through 20 generations in medium C containing minced chick embryo, Tyrode's solution and normal rabbit serum. In the same medium cultures of the Webster strain were virulent through 8 generations, beyond which point further cultivation was not attempted. The Barnes strain was grown in one experiment through 11 successive transplants. Subcultures were continued to the 14th transfer but the last two passages failed to infect animals. The 5th culture generation of this series failed to infect mice, but infections with material from the 6th were typical. Another series of cultures of this strain was discontinued after the 4th subculture although virus was demonstrated at that time. In D, similar medium without serum, the Daily and Barnes strains were transplanted for 9 generations and the Webster strain through 8 generations. The last culture in each case was lethal for mice.

Cultures in medium A were initiated with infected mouse brain. Most cultures in other media were inoculated with culture virus, and the total number of generations *in vitro* was actually greater than is indicated above. These figures are recorded in Table I.

Titration to determine the quantity of virus present in the cultures were carried out on the 6th and 22nd generations of the Daily strain and on the 6th culture passage of the Barnes strain.

The Barnes strain cultures, after incubation for 5 days, were diluted  $10^{-1}$ ,  $10^{-2}$ ,  $10^{-4}$  and  $10^{-6}$  and then injected into four groups of four mice each. This culture virus was lethal for all mice receiving  $10^{-1}$  and  $10^{-2}$  dilutions. Higher dilutions had no effect as measured by the presence of clinical symptoms or immunity on subsequent inoculation of mouse passage virus.

Titration of the 6th culture passage of the Daily strain was performed in the identical manner employed with the Barnes titration. The mice receiving dilutions of  $10^{-1}$  and  $10^{-2}$  died on the 5th and 6th days after inoculation. None of the animals injected with the  $10^{-4}$  dilution gave evidence of infection, but two of the group receiving the highest dilution ( $10^{-6}$ ) died after a longer incubation period. When the six survivors were tested at a later date by intracerebral injection of mouse passage virus two were found to be immune.



The 22nd generation of the Daily strain was titrated at various time intervals (1, 2, 3, 4, 6, 7, 10 and 17 days after inoculation of the flasks) to determine the effect of incubation time on the potency of the virus. Samples tested from the 1st to the 10th day regularly killed mice at a dilution of  $10^{-2}$ , and in some tests (1, 4, 7 and 10 days) killed irregularly at a dilution of  $10^{-3}$ . There was a distinct decrease in the amount of active virus present after 17 days as evidenced by death of only two of three mice receiving undiluted culture and of only one of three injected with the  $10^{-1}$  dilution. Another set of cultures of the same strain was virulent for mice after incubation at  $37^{\circ}$  C. for 5 days, but not after 29 days.

Titration determining the amount of virus in inoculated culture media of this type indicate that a  $10^{-2}$  dilution is uniformly lethal for mice and that higher dilutions irregularly kill mice or immunize them to subsequent infection by inoculation. Further, although the titer of culture virus was much lower than that of virus in the brain of passage animals, the data show that there was no significant decrease in potency during the 16 culture passages intervening between two sets of titrations of one (Daily) strain.

#### *Cultures with Immune Serum*

An attempt was made to cultivate St. Louis encephalitis virus on mouse embryo tissue in the presence of rabbit serum containing neutralizing antibodies against the virus. Control cultures were grown in mouse embryo medium with normal rabbit serum, test cultures in the same medium with immune serum. Fresh mouse passage virus (Daily strain) was used for inoculum. Cultures were transplanted at intervals of 4 or 5 days into the same kinds of medium. In addition, each culture in immune serum was subcultured in medium containing normal serum by transplanting whole culture material in some cases, in others tissue fragments which had been centrifugated and resuspended in Tyrode's solution in order to remove most of the immune serum.

Culture material from both control and test cultures was washed to remove the serum before inoculation into mice. All mice injected with material from control cultures in normal serum medium died. None were affected by material from immune serum cultures or from 1st, 2nd or 3rd subcultures of these in control medium, and all the survivors succumbed when subsequently inoculated with mouse

passage virus. Thus the virus either failed to survive in the presence of immune serum or was modified to such an extent that it failed to infect or immunize mice. The experiment was discontinued after 4 culture passages.

### *Cultivation in Vivo*

Two strains of St. Louis encephalitis virus were inoculated in series in 13 to 16 day old chick embryos. The embryos were often alive and active 5 or 6 days after infection with virus and occasionally hatched normally. However, the majority died on the 4th or 5th day after injection.

Three attempts were made to propagate the Barnes strain in serial passage in eggs. In each case the initial inoculum consisted of a suspension of ground tissue from a flask culture. One experiment was terminated early by bacterial contamination. In another the original inoculum proved to be non-infectious for mice but passage was continued for a number of generations as a control series. Mice inoculated with material from embryos and membranes in this series remained alive and apparently unaffected by the foreign tissue. The third experiment was continued in duplicate series through 9 egg passages.

Four series of egg passages were begun with the Daily strain. In two earlier series in which younger embryos were used the virus was lost by the 3rd passage. Another series was discontinued after a few passages although the virus was still potent. The fourth series was continued in two parallel experiments through 10 passages.

As indicated in Table II, the Barnes strain of the virus was of maximum infectivity for embryos and mice in each of the first 7 passages. It was recovered from the brain as well as the membranes of the 4th and 6th passage embryos. Mice injected with material from 8th passage embryos died apparently of bacterial infection. There were no lesions in the chorio-allantoic membranes of the 9th passage embryos, and mice injected with tissue from them were not infected.

The Daily strain was recovered from embryos in each of 10 serial passages of the virus by inoculation of mice with chorio-allantoic tissue. Mice died with typical encephalitis also after inoculation with brain, liver and spleen of an embryo in the 7th passage, and with liver from an embryo in the 9th passage.

TABLE II

*Growth of St. Louis Encephalitis Virus in Developing Chick Embryos*

Virus strain	Egg passage †	Embryos used for egg passage and mouse inoculation				Results of mouse inoculation *	
		Age in days when inoculated	Days of incubation after inoculation	Condition of embryos	Character of lesions in chorio-allantoic membranes	With chorio-allantoic membranes	With other embryo tissues
Barnes	1	14	3	1 alive	Proliferative	5, 5, 5	
			7	1 dead	Large, necrotic center	5, 7, 7	
			8	1 hatched paralyzed			
	2	15	6	1 alive	Large, necrotic center	6, 6, 7	
			2	1 dead	Cloudy patches	6, 6, 6	
	3	15	7	1 dead	Large, necrotic center	6, 6, 6	
	4	12	10	2 dead	" " "	6, 6, 7	7, 7, 8 (brain)
	5	14-16	4-6	3 dead 1 hatched	" " "		
				2 dead	" " "	4, 5, 7	
	6	14	6	1 alive	" proliferative	6, 6, 6	6, 6, 6 (brain)
	7	14-15	4-6	2 dead	" necrotic center	6, 6, 6	
	8	15	6	2 dead	" necrotic	2, 2, 2 ‡	
			5	1 alive	" "		
	9	15	4	1 alive	" "	s, s, s	s, s, s (liver)

	1	15	4	1 alive	Proliferative	5, 6, 6	
	2	13-14	5	1 dead 1 alive	"	6, 6, 6	
	3	13	5	1 alive	"	1, 7, 7	
	4	14	5	1 dead	"	6, 6, 8	
	5	12-16	7-9	2 dead	Large, necrotic center	7, 7, 8	
	6	13-15	6	2 dead	Proliferative	6, 6, 6	
	7	14	4-6	2 alive	Large, necrotic center	6, 6, 7	6, 6, 6 (liver and spleen) 6, 6, 6 (brain)
	8	14	4	1 dead	" " "	1, 5, 5	
		14-15	4	2 alive	" " "	6, 6, 6	
	9	16	4-5	2 alive	" " "	4, 6, 6, 8	6, 6, 7 (liver)
	10	12-15	3-4	2 alive	" " "	5, 5, 6, 7, 7, 8	

\* Three or more mice were inoculated intracerebrally with each tissue suspension. Figures represent dates of death. s = survived.

† Six or more eggs inoculated for each passage.

‡ These mice apparently died of bacterial infection.

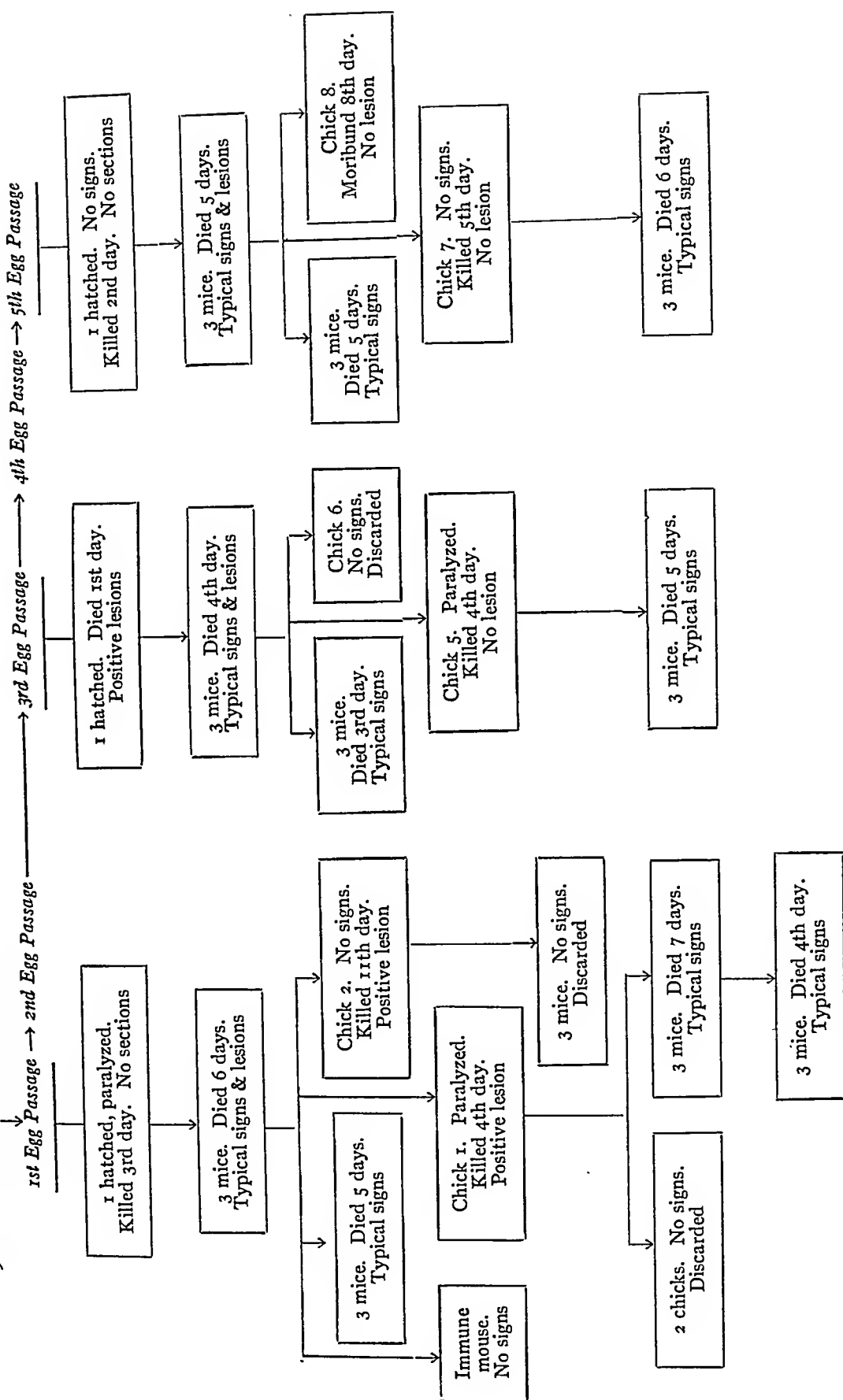
*Changes Observed in Infected Chick Embryos*

The pathogenicity of St. Louis encephalitis virus for the chick embryo was evidenced by the lesions of the chorio-allantoic membranes and by frequent death of the embryos. The lesion of the chorio-allantoic membrane appeared first as an opacity, then a thickening, and finally, if the embryo lived long enough, a definitely necrotic patch at the site of inoculation. Often the gray patch of proliferation spread out beyond the triangular area under the window, but was of maximal intensity at that site. Microscopically a proliferation of all three germ layers was observed. Early lesions consisted mainly of proliferation of the ectoderm, but as time went on the other two layers became involved. The ectoderm became thickened to a depth of six to eight cells, with the formation of epithelial pearls. Proliferation of the endoderm was much less pronounced. In the mesoderm an increase of fibroblasts and an invasion of epithelial cells were seen, and often large mononuclear cells were grouped around vessels. In some cases there was an exudate on the ectodermal surface, composed of polymorphonuclear and mononuclear cells with pyknotic nuclei and many erythrocytes. When an exudate existed on the ectoderm there was generally a sprinkling of polymorphonuclear cells and erythrocytes throughout the mesoderm. The vascularity of the ectoderm was greatly increased. No cell inclusions were seen and no pathological changes were found in brains of infected embryos, including those from which virus was recovered by inoculation of mice with brain tissue, although, as described below, lesions were encountered in the brain of a chick that hatched after inoculation.

*Infection of Young Chicks*

One egg in each of the 1st, 2nd, 3rd, 5th and 6th passages of the Barnes strain was incubated until it hatched. The chicks from the 2nd and 6th passages died while hatching and were discarded. Brain tissue from the others was used for inoculation of mice and young chicks in the experiments recorded in Text-figure 1. The chick hatched from a 1st passage egg, paralyzed in both legs, was killed after 3 days and a 10 per cent suspension of its brain was inoculated intracerebrally into mice. These developed convulsions and were killed when moribund 6 days after inoculation. The micro-

10th Culture Generation in Medium A



Text-figure 1. Passage of St. Louis encephalitis virus (Barnes strain) through young chicks.

scopic lesions observed in the brain were typical of those caused by the virus. Brain emulsion from these mice inoculated into three normal mice induced typical convulsions and death on the 4th and 5th days, while a similar inoculation in a mouse recovered from a previous infection with the Daily strain was without effect. The same mouse brain suspension was also inoculated intracerebrally into two young normal chicks, 4 to 6 days old. One of these remained well and the virus was not recovered when it was killed 10 days later, despite the fact that lesions were found in sections of the brain. The other became partially paralyzed and was sacrificed 4 days after inoculation. Brain suspension from it induced no symptoms in two young chicks but caused death in mice within 7 days. Virus recovered from them killed a second set of mice on the 3rd and 4th days. This experiment was not continued further.

The chick obtained from the 3rd egg passage died on the day it emerged from the shell. The fifth, apparently normal, was sacrificed for study on the 3rd day. Brains from both these chicks were used for passage of the virus through mice and young chicks. As indicated in Text-figure 1 the results were similar to those described above. In each case the virus was recovered from two sets of mice, inoculated serially, and from chicks.

Thus, the St. Louis virus, recovered from the brain of each of three newly hatched chicks, which had been inoculated on the chorio-allantoic membrane 5 to 8 days before hatching, was passed in series through mice and young chicks. Microscopic pathological changes were seen in the brain of a hatched chick from the 3rd egg passage, and in two normal chicks that were inoculated, after hatching, with mouse brain infected with 1st egg passage virus. They consisted of extensive perivascular cuffing with mononuclear cells and an occasional small glial nodule but no meningeal reaction. One chick in each of the other two series exhibited clinical signs of infection but no pathological changes were observed in sections. Further chick to chick passage in the first series produced no signs or lesions, although the virus was present in the passage material as demonstrated by mouse inoculation. Mice inoculated with brain emulsions from infected chicks exhibited clinical and pathological pictures characteristic of St. Louis encephalitis.

The occurrence of paralysis and pathological changes in the brains of certain chicks indicate a potential susceptibility of young chicks

to the virus. Also, there is a suggestion that chicks may carry the virus without manifestation of infection. It is interesting in this connection that virus was not recovered from Chick 2, sections of which showed encephalitic changes.

## DISCUSSION

Three strains of the virus of St. Louis encephalitis have been cultivated *in vitro* in media composed of minced mouse embryo tissue suspended in Tyrode's solution, either with or without normal rabbit serum, but the virus failed to survive when immune serum was present. Two different strains of virus were infectious for mice in the 25th and 26th subcultures *in vitro* when observations were discontinued. These final generations represented an approximate dilution of  $10^{-26}$  and  $10^{-27}$ , respectively, of the original infected mouse brain. Since the approximate final dilution in cultures was far beyond the end point of infectivity of the original mouse brain,<sup>10</sup> and since virus kept under conditions not favorable to growth becomes inactive within a few hours,<sup>11</sup> propagation of the virus *in vitro* undoubtedly occurred.

The experiments reported here agree in general with the observation of Syverton and Berry<sup>3</sup> that the infectious titer of culture virus is in the neighborhood of  $10^{-2}$ . Although this titer is definitely lower than that occurring in routine mouse passage virus it was maintained over a period of at least 22 culture generations. Cultivation of the virus *in vitro* does not appear to alter its characteristic effects on mice, as it induced the usual clinical signs and pathological changes after an incubation period typical of mouse passage virus.

The virus was successfully cultivated also in similar media containing chick embryo tissue and in developing chick embryos. It induced changes in the chorio-allantoic membranes and remained virulent for mice through 10 serial transplants in the fertile egg. Virus was recovered from the brain, liver and spleen, as well as from the chorio-allantoic membranes of infected chick embryos and from the brains of chicks that hatched after being infected. Since the 10th egg passage material represented a dilution of the original inoculum of about  $10^{-32}$  it may be assumed that the virus actively multiplied in the chick embryo.

Although mature chickens are not affected by the virus<sup>10</sup> very



young chicks were found somewhat susceptible. Half of the small number used showed clinical or pathological changes, or both, and virus was recovered in all but 1 of the passages from chick to mice.

The modified neurotropism of the St. Louis virus, demonstrated by Webster and Clow<sup>12</sup> in mouse experiments, is further exemplified in the present observations. Histological changes in all three germ layers of the chorio-allantoic membrane, together with the isolation of virus from the liver and spleen of developing chick embryos, confirm and extend the observations of Webster and Clow concerning the viscerotropic action of the virus.

### SUMMARY

1. Three strains of the virus of St. Louis encephalitis have been successfully cultivated *in vitro* in the presence of living cells.
2. The media employed were minced mouse or chick embryo tissue suspended in Tyrode's solution, either with or without rabbit serum.
3. Media containing normal rabbit serum supported the growth of virus better than media without serum. When rabbit serum possessing neutralizing antibodies against the virus was employed in cultures the infectious agent was not recovered.
4. Two strains of the virus were propagated in serial passage in the developing chick by inoculation on the chorio-allantoic membrane. The virus was recovered from the brain, liver and spleen, as well as from the chorio-allantoic membrane of passage chick embryos. Histopathological changes occurred in all three germ layers of the embryonic membrane.
5. Young chicks are to some extent susceptible to the virus.

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# FIBROSIS OF THE BONE MARROW (MYELOFIBROSIS) ASSOCIATED WITH A LEUKEMOID BLOOD PICTURE \*

## REPORT OF TWO CASES

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In 1879 Heuck<sup>1</sup> described a case of leukemia in which the bone marrow at autopsy was found encroached upon by a network of fibrous tissue and by fine bony trabeculations. This condition was later classified as osteosclerosis by Kaufmann in his textbook of special pathological anatomy. From time to time other cases of osteosclerosis have been reported in the German literature. The subject was well reviewed by Oesterlin<sup>2</sup> in 1924, by Mavros<sup>3</sup> in 1931, and by Anagnostu<sup>4</sup> in 1933. More recently similar cases have been reported in this country by Stephens and Bredeck<sup>5</sup> and Chapman.<sup>6</sup>

Following the early publications of Heuck,<sup>1</sup> von Jaksch,<sup>7</sup> Askanazy,<sup>8</sup> and Nauwerck and Moritz,<sup>9</sup> two types of osteosclerosis were recognized: the so-called marble bone or Albers-Schönberg<sup>10</sup> disease, and myelosclerosis or myelofibrosis. In the former type the essential lesion is one primarily of excessive bone formation, with gradual thickening of the cortex and encroachment on the medullary cavity. The condition occurs usually in childhood, persists throughout life into adulthood and terminates with a myelophthisic anemia. The latter type of the disease occurs most often after the second decade, and a leukemoid blood picture with enlargement of the spleen and severe anemia are the outstanding clinical findings. The pathological process in the medullary cavities consists of fibrosis with some bone formation.

The case reports which follow are, we believe, of the latter type, presenting leukemoid blood pictures and fibrosis of the bone marrow.

\* Received for publication October 5, 1936.

## CASE REPORTS

CASE 1.\* A. A. W., a white American housewife, aged 40 years, consulted her physician on July 14, 1923, because of pains in the left arm and the legs, and the appearance of purpuric spots in the skin. She stated that she had been "pale and anemic" since childhood and had received injections of iron for this condition. However, an examination of her blood by a physician on Dec. 4, 1919, had shown that the red blood cells were 4,280,000 in number, with a hemoglobin reading of 88 per cent. On Feb. 14, 1922, examination of the blood had given approximately the same results. Six months prior to visiting her physician an operation had been performed for suspension of the uterus. Bleeding was not unduly notable at that time. At the time of operation the hemoglobin was reported to be 85 per cent. In May, 1923, a sharp, stabbing pain occurred in the left arm. More recently a similar pain developed in the knees and the upper portion of the sternum. During the month of May the appearance of purpuric spots was noticed in the skin, there was a tendency to bleed from the nose, and the menstrual flow was prolonged. At times there had been bleeding from the gums, but blood had not been seen in the urine or feces.

*Physical Examination:* In July, 1923, extreme pallor of the skin and mucous membranes and the presence of many small purpuric spots in the skin was noted. There was no evidence of organic heart disease or infection in the ears, the throat or about the teeth to account for her condition. There was no demonstrable enlargement of the spleen, liver or superficial lymph nodes.

*Laboratory Data:* Examination of the blood showed: red blood cells 2,080,000 per cmm., hemoglobin 43 per cent, and white blood cells 4200 per cmm. The differential count was: neutrophiles 22 per cent, myelocytes 43 per cent, unclassified cells 16 per cent, lymphocytes 15 per cent, and monocytes 4 per cent. The 16 per cent unclassified cells were thought probably to be myelocytes. An occasional normoblast was seen. The blood platelets were obviously reduced in the blood smear. A specimen of blood clotted in  $1\frac{1}{2}$  minutes but failed to retract. Bleeding from a small puncture of the ear continued after 7 minutes.

*Course of Illness:* During the following months, from July to December, the patient's hemorrhagic symptoms were ameliorated by transfusions of whole blood, given at intervals of every 3 to 4 weeks. She continued, however, to have a persistent leukopenia and an anemia, and at times the platelets were extremely scant in the blood smear. The spleen was not found enlarged by palpation, nor did it cast an abnormally large radiographic shadow until November.

In December, 1923, splenectomy was performed and the operation was followed by a rise in the platelet count from approximately 10,000 to 100,000 per cmm. Following operation the gums bled slightly for a few days and then all bleeding stopped. The patient showed general symptomatic improvement until March, 1924, when the purpuric spots, joint pains and bleeding of gums returned.

On March 25th examination of the blood showed: red blood cells 2,400,000, hemoglobin 36 per cent (Sahli), color index 0.75, and white blood cells 25,900. The differential count was: neutrophiles 10 per cent, lymphocytes 31 per cent, monocytes 5 per cent, and myelocytes 54 per cent. Her condition became rapidly worse and she died on March 29, 1924.

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\* The authors wish to thank Drs. Fletcher Taylor, George Evans and Ernest Falconer for permission to use the clinical data on this case.

*Autopsy Abstract\**

The body appeared well developed and fairly well nourished. There was extreme pallor of the skin and mucous membranes. In the skin over the chest, abdomen, arms and legs there were numerous areas of petechial hemorrhage measuring up to 3 mm. in diameter. The gums were spongy and soft with bleeding surfaces. The neck showed no palpable lymph nodes or enlargement of the thyroid. The abdomen was considerably distended. Along the left costal margin was a long scar from the surgical incision for splenectomy. Between the umbilicus and the symphysis pubis was found another linear scar of an old operation. There was a moderate amount of fat in the abdominal wall. On opening the peritoneal cavity a few, dense fibrous adhesions were seen between the omentum and abdominal wall, beneath the diaphragm, and about the stomach in the region from which the spleen was removed.

*Heart:* The heart weighed 270 gm. There were three small areas of petechial hemorrhage beneath the epicardium and a few beneath the endocardium of the right auricle.

*Lungs:* The lungs showed nothing noteworthy except slight emphysema.

*Liver:* The liver weighed 2070 gm. Fairly fresh adhesions were found over the superior surface of both lobes and rather dense adhesions involved the region adjacent to the stomach. The tissue was extremely pale and somewhat flabby. The cut surface was smooth and glistening and showed several, minute, circumscribed whitish areas. The lymph nodes at the hilus of the liver were greatly enlarged, soft and hyperemic.

*Gall-bladder:* Normal.

*Pancreas:* Normal.

*Spleen:* The spleen was missing on account of operative removal.

*Stomach:* The stomach was distended and filled with a watery, slightly hemorrhagic fluid.

*Ileum:* There was a diffuse hemorrhage beneath the mucosa and serosa in the wall of the ileum.

*Kidneys:* The combined weight of the kidneys was 320 gm. The capsules stripped readily, leaving an exceedingly pale surface which showed the remains of fetal lobulation. Numerous petechial hemor-

\* Autopsy No. A-24-87.

rhages were found on the surfaces. On cut section nothing of note was found except marked pallor.

*Uterus and Adnexa:* The uterus was approximately normal in size. There were some old, healed, irregular lacerations of the cervix. The appendix, right Fallopian tube and ovary were absent as a result of surgical removal. The left ovary contained three large cystic areas filled with clotted blood.

*Bone Marrow:* The marrow of the ribs and of the femur was of a pale grayish color. There was a large amount of cancellous bone and fibrous tissue which extended throughout the medullary portions of the bones.

### *Histological Examination*

Examination of the thyroid, lungs, heart, kidneys, pancreas, intestines and adrenals was consistent with the characteristics noted by gross inspection.

*Liver:* A section of liver showed scattered, miliary-like foci of cells which were chiefly of a large mononuclear type. The nuclei were large and contained an abundance of chromatic material enclosed in a definite nuclear membrane. The nuclei were surrounded by varying amounts of acidophilic cytoplasm. Comparatively large numbers of a similar type of cell were to be seen in the sinusoids. No foci of erythropoiesis were seen. In the liver cells about the periphery of the lobules a deposition of golden brown pigment was found.

*Lymph Nodes:* A section of a retroperitoneal lymph node showed definite alteration of its architecture. No germinal centers were seen. The tissue was moderately cellular and composed mostly of small mononuclear cells resembling lymphocytes. Interspersed among these, however, were seen large mononuclear cells, each containing a large round nucleus. The nuclear membrane was well defined and surrounded a sparse amount of chromatic material. The cytoplasm about these nuclei varied considerably in amount in individual cells stained with eosin. The type of cell seemed to be of myeloid origin. An occasional large multinucleated cell which resembled the megakaryocytes of the bone marrow was also seen. The sinusoids were greatly dilated and contained large numbers of mononuclear cells which presumably were myelocytes. In the sinusoids were to be seen an occasional megakaryocyte. There was no evidence of erythropoiesis.

*Bone Marrow:* A specimen of bone marrow removed from the femur showed tissue made up of blood formative cells and fibrous tissue. In some areas were myelocytes and myeloblasts forming compact hyperplastic foci of leukopoiesis. Elsewhere the cells were loosely arranged and scattered. Only occasional small foci of erythropoiesis were noted. A loose connective tissue extended throughout the section of bone marrow between small spicules of bone. In this connective tissue were large numbers of fibroblasts. This tissue was moderately vascular. No megakaryocytes were seen.

A specimen of bone marrow removed from the rib showed tissue that was, in general, more cellular than the femoral marrow; however, the connective tissue reaction was greater and in some places completely replaced the marrow elements. Proximal to the cancellous portion of the bone, in some areas, dense strands of wavy connective tissue fibrils were to be seen. In such areas there was but scant evidence of hematopoiesis.

*Spleen (Surgical Specimen):* The architecture was loose in structure and supported a moderately cellular pulp. Numerous cells of the myeloid series were to be seen and suggested metaplasia consistent with the diagnosis of leukemia. In the sinusoids large mononuclear cells resembling myeloblasts were to be seen. The malpighian bodies of the spleen appeared to be present in normal size and numbers. There was no evidence of erythropoiesis.

CASE 2. A. M., an American male, aged 34 years, married, was admitted to the Mount Zion Hospital on April 25, 1935, complaining of general malaise, weakness, fever and sweating of about 2 weeks duration. He first came to the Clinic in May, 1932, at which time a diagnosis of chronic myeloid leukemia was made, and since that time he had been under observation at frequent intervals. Because of a high white cell count, irradiation by roentgen ray had been administered between May and October, 1932, and he had received a total of 800 R over the spleen and abdomen. In 1933, during the course of the year he had received four treatments totalling 400 R. In 1934 he had received a total of 630 R over the abdomen. During 1935, from February to the time of death, 5 months later, he received 500 R over the abdomen and extremities.

Two weeks prior to entry in the hospital he began having afternoon chills followed by profuse diaphoresis. There was no pain but at times he complained of generalized "body ache."

*Physical Examination:* On entry to the hospital the patient was well developed but undernourished and appeared quite ill. The temperature was 39.5° C. (102.9° F.); the pulse rate was 124; and the respiratory rate 28. The mucous membranes were pale and the skin moist. The ears, teeth, nose, throat and lymph nodes presented no remarkable abnormalities. There was no evidence of cardiac disease. The blood pressure was 120 systolic and 70 diastolic. Extreme



tenderness was elicited on pressure over the sternum. The edge of the spleen was felt 4 cm. below the left costal margin, and the edge of the liver 3 to 4 cm. below the right costal margin.

*Laboratory Data:* The laboratory findings, with the exception of the blood counts which are recorded in Table I, were essentially negative. A sample of venous blood obtained on May 27, 1935, was reported as yielding no growth of organisms.

*Clinical Course and Treatment:* The patient did not show much change in his condition until May 18, 1935, when he suddenly became extremely weak and dyspneic. On this date he was given a transfusion of 500 cc. of whole blood. The following day the patient complained of impaired vision in the right eye. On ophthalmoscopic examination numerous small hemorrhages were seen in each fundus. On May 23, 1935, he received another transfusion of 500 cc. of whole blood. Despite this treatment the course of the patient's illness was progressively downhill. On May 31, coarse and crepitant râles became audible in both lungs, and on this date the patient expired.

### *Autopsy Abstract*

The body was that of a white adult male appearing to be about 35 years of age, poorly nourished but normally developed. Extreme pallor of the skin and mucous membranes was present. There was no enlargement of lymph nodes in the neck or popliteal spaces, but a few enlarged lymph nodes were found in the axillae. The liver was palpable in the right upper quadrant. The external genitalia were normal, as were the extremities. The usual midline incision showed a normal peritoneal cavity. The liver edge extended two finger-breadths below the costal margin, and the spleen was greatly enlarged. The pleural and pericardial cavities appeared normal. No abnormal lymph nodes were found in the mediastinum, mesentery or retroperitoneum.

*Heart:* The heart was of normal size and shape. There was no evidence of endocarditis.

*Lungs:* The lower lobes of both lungs were "boggy" and congested because of a bronchopneumonia.

*Gastro-intestinal Tract:* The esophagus was normal. There were some minute discolored foci in the mucosa of the stomach, suggestive of ulcerations. The small and large intestines were normal.

*Trachea:* There were no gross changes in the true and false vocal cords.

*Liver:* The liver was about one and a half times normal size, soft and uniform in consistence, and a light brown.

*Spleen:* The spleen was about twice normal size, firm in consist-

TABLE I

*Data Showing the Progressive Development of Severe Anemia, Thrombocytopenia, and the High Percentage of Immature Leukocytes in the Differential Formulas Reported in Case 2*

Date	Hemoglobin (Sahli)	Red blood cells in mil- lions per cmm.	Platelets per cmm.	White blood cells per cmm.	Polymorphonu- clears		Poly- morphonu- clear eosino- philes	Poly- morphonu- clear baso- philes	Lym- pho- cytes	Mono- cytes	Myelo- cytes	Myelo- blasts	Normo- blasts per 100 W.B.C.	Treatment
	%				Fila- mentous	Non-fila- mentous								
5/11/32	85			68,500	16	51	4	1	8	1	18	1	2	X-ray started To date received 800 R
10/27/32	11.9 gm. 90	4.95		11,500	47	18	4	3	23	4	1			
1/13/33	93 14.4 gm.	5.44	320,000	16,800	55	12	4	6	17	5	1			400 R
12/12/33	86 13.4 gm.			17,750	50	19	2	6	11	6	6			
4/3/34	88 13.6 gm.			26,600	37	25	4	4	16	6	8			630 R
12/29/34	54 8.3 gm.			10,400	71	3	1	5	11	5	4			
4/19/35	48 7.6 gm.	2.57	100,000	26,250	12	39	2	2	4	4	31	6		1% ? megakaryocyte 1% ferrata cell Entered hospital
4/26/35	28 4.4 gm.	1.61	135,000		7	45	4	1	15	1	12	15	3	
5/21/35	33 5.2 gm.	1.97		7,500	2	11			38	3	1	45	3	Transfusions 2 X-ray 500 R
5/27/35					0	5			24	3	6	62	7	

ence and a uniform maroon red. On cut section the normal splenic parenchyma appeared to be replaced by a reddish cellular material. The malpighian corpuscles and the fibrous trabeculations were not evident.

*Pancreas:* The pancreas appeared normal.

*Kidneys:* The kidneys were slightly enlarged and equal in size. The capsule stripped with ease, showing a normal smooth surface. On cut section the cortex and medulla were found to be sharply demarcated. The pyramids stood out and there was no evidence of leukemic involvement of the parenchyma. The adrenals, ureters, bladder and prostate were apparently normal.

*Bone Marrow:* The marrow of the femur was firm, red, and appeared to be hyperplastic.

### *Histological Examination*

Examination of heart, stomach, pancreas, kidneys, prostate, aorta, adrenals and thyroid tissue showed nothing of significance.

*Liver:* Microscopic examination of the liver showed the presence of a normal architecture. Within the liver cells, especially about the periphery of the lobules, was a deposition of yellowish brown pigment. The Kupffer cells were approximately normal in size, although occasional cells showed considerable hypertrophy. Many of these cells were filled with yellowish brown pigment. The sinusoids showed an unusually large number of cells, the majority of which were large in size, and each contained a large round nucleus. The nuclei for the most part showed a distinct membrane and contained one or more clumps of deeply stained basophilic chromatin. The cytoplasm of these cells varied in amount from a thin rim up to a rather abundant, irregularly shaped mass. The cytoplasm showed faint staining with eosin. Among these cells was an occasional mitotic figure. These were all immature types of cells of the leukopoietic series, and apparently in the myeloblast and promyelocyte stages of development. The adult forms of polymorphonuclear leukocytes were rarely seen. Both the large and small forms of lymphocytes were apparently reduced in number. The red blood cells were comparatively sparse in number; many of these showed considerable anisocytosis and poikilocytosis and seemed to contain a decreased amount of hemoglobin. Normoblasts were exceedingly few in number. An occasional, large mononuclear type of cell, with

an abundant, irregularly shaped cytoplasm was noted. Within the cytoplasm of such cells were seen phagocytosed red blood cells and brown pigment. There were no accumulations around the periportal spaces.

*Testes:* There were two or three areas of accumulations of immature leukocytes, with an occasional polymorphonuclear.

*Spleen:* The essential structure was still present but the finer details appeared somewhat disrupted by the presence of numerous unusual cells throughout the pulp. These cells were large, and each contained the large nucleus that often appears in mitotic configuration. Only an occasional adult leukocyte was noted. There was no evidence of erythropoiesis. The malpighian follicles were small and the trabeculae thin. There was no unusual formation of fibrous tissue.

*Bone Marrow of Femur:* The usual marrow structure was entirely replaced by a cellular fibrous tissue in which were scattered hematopoietic cells. In the more cellular areas, of which there were few, the fibrous tissue was rather loose in structure. The leukopoietic tissue was mainly represented by a large type of cell such as was found in the liver and spleen. This presumably was a myeloblast. Myelocytes and an infrequent small group of normoblasts were present. Mitotic figures were noted frequently throughout, especially in the large undifferentiated cells. No megakaryocytes were to be found.

*Costal and Sternal Marrow:* There appeared to be a combination of an extensive fibrosis with scattered persisting areas of hematopoiesis in the costal and sternal marrow.

*Trachea:* A specimen of marrow from a bone in the trachea showed a similar pattern of fibrous tissue but was slightly more cellular.

## DISCUSSION

The clinical findings in the 2 cases reported here are similar only in the qualitative aspects of the so-called "leukemoid" blood pictures. The illness of the first patient described was characterized by a hemorrhagic tendency, thrombocytopenia and severe anemia. Enlargement of the spleen occurred late in the course of illness. At the outset a leukopenia occurred and immature forms of leukocytes were found in the blood smear. Prior to death a leukocytosis de-

veloped, and concomitantly there was a definite increase in the number of immature leukocytes. In the second patient the classical findings of myelogenous leukemia were encountered when the patient first sought medical aid. There was splenomegaly and leukocytosis, and myelocytes were present in the blood film. As the course of illness approached termination, myeloblasts appeared in the blood and the patient developed in addition a severe anemia.

On postmortem examination these 2 cases presented a striking similarity in the structural changes of the long bones. This is shown in Figures 1, 2 and 3, where it is to be noted that the medullary cavities showed increased fibrosis and a few spicules of cancellous bone which partially obliterated the hematopoietic tissue.

In the 1st case, substantiation for the diagnosis of leukemia was found elsewhere in the body, as evidenced by the extramedullary proliferation in the spleen, liver and lymph nodes. The pronounced alteration of the lymph nodes was perhaps the most characteristic feature of leukemia. The normal architecture was largely obliterated. Myeloid cells in various stages of maturation were present in the sinusoids and uniformly scattered among the remaining lymphoid cells. Megakaryocytes were occasionally seen. In the 2nd case, extramedullary leukopoiesis was of lesser degree, and there was neither apparent involvement of lymph nodes nor periportal infiltration in the liver. The sinuses of the liver and spleen were, however, filled with cells of a type transitional between myeloblasts and adult forms.

Extramedullary erythrocytopoietic activity has been described in some cases of myelofibrosis. A careful study of the sinusoids of the liver and spleen in our 2 cases, however, failed to reveal any erythro-genetic foci.

In the 1st case reported here the blood picture was compatible with a diagnosis of aleukemic myelosis, whereas in the 2nd case the findings were those typical of myeloid leukemia.

Stephens and Bredeck,<sup>5</sup> in their review of the literature up to 1932, were able to report 19 cases of fibrosis of the bone marrow. All of these cases were consistent with a diagnosis of leukemia. The leukocyte counts varied from as low as 5200 to 80,000 per cmm., and about one-third of these could be designated as aleukemic. Myelocytes and normoblasts were found in the blood smears, and there was enlargement of the spleen in all cases. Since their report a

few other cases with a leukemoid blood picture have been recorded in the literature. Anagnostu <sup>4</sup> described a case of systemic osteosclerosis in a male aged 65 years who had an anemia, a leukocyte count of 3900, and promyelocytes and myelocytes in the blood film. The spleen was moderately enlarged. The combined clinical and postmortem diagnosis was aleukemic myelosis with bone marrow fibrosis. Two cases of osteosclerosis are reported by Chapman <sup>6</sup> in which splenomegaly, anemia and leukopenia occurred, and immature forms of leukocytes were found in the blood film. In these cases biopsy of the sternal marrow showed the presence of fibrosis and established the diagnosis.

There is considerable discussion in the literature as to the exact relation of the leukemoid blood picture to the marrow fibrosis and osteosclerosis. The changes have been thought by some observers to be similar to those occurring in myelophthisic anemia associated with a carcinomatous metastasis to the bone marrow. This conception would presuppose that some initial infection or bone marrow injury had led to the fibrosis, and that the resulting blood changes were secondary to this process. On the other hand, the comparatively large number of cases showing manifest leukemia and a high white count suggests that the fibrosis and bony overgrowth occur secondary to the marrow hyperplasia and may represent the desmoplastic reaction that is characteristic of some cases of carcinoma.

### SUMMARY

Two cases of fibrosis of the bone marrow associated with leukemoid blood pictures are presented. In the 1st case the onset of illness was characterized by leukopenia and a hemorrhagic tendency, and later with moderate enlargement of the spleen and a terminal leukocytosis. The course of illness in the 2nd case was typical of leukemia for a period of 3 years prior to the patient's death. In both cases during the postmortem examination there were found in the liver, spleen and lymph nodes changes compatible with a diagnosis of leukemia, but there was partial obliteration of the marrow cavities with fibrous tissue and bony spicules.

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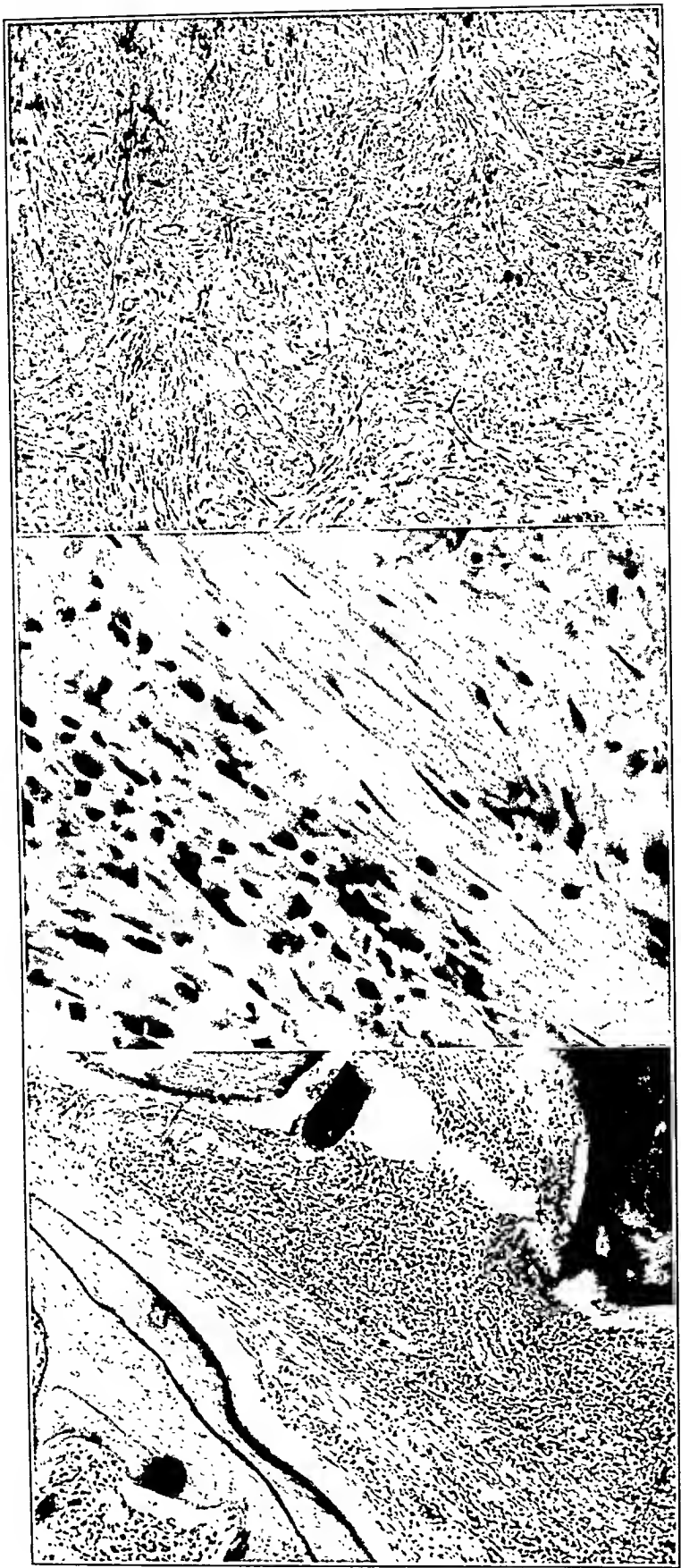
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DESCRIPTION OF PLATE

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PLATE 59

- FIG. 1. Case 1. Showing partial fibrosis of a specimen of femoral bone marrow removed at autopsy.  $\times 110$ .
- FIG. 2. Same as Figure 1, but higher power.  $\times 250$ .
- FIG. 3. Case 2. Showing extreme fibrosis of a specimen of femoral marrow removed at autopsy.  $\times 110$ .







# THE AORTIC COMMISSURAL LESION IN RHEUMATIC FEVER \*

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One of the most conspicuous gross lesions in the rheumatic heart is the commissural agglutination of the aortic cusps. In its characteristic form it consists of an adhesion of adjoining sigmoid cusps which extends for a variable distance toward the center of each involved leaflet but preserves the groove separating the cusp edges. In this respect, therefore, the rheumatic commissural lesion of the aortic valve differs grossly from the generally rounded, smooth broad bridge of healing or healed subacute bacterial endocarditis, from the irregularly widened and eroded lesion of active subacute bacterial endocarditis, from the nodular and distorted fusion of the Mönckeberg type of aortic valve calcific sclerosis, from the separated margins of the syphilitic commissure, and from the depressed and almost obliterated raphe considered characteristic of the so-called congenital bicuspid aortic valve.

In spite of the striking picture presented by the rheumatic commissural lesion no reports are available on its pathogenesis. The present study concerns itself with this problem, together with the question as to whether or not, as in other cardiac sites (Gross *et al.*,<sup>1-9</sup>), the different clinical courses that rheumatic fever may take are reflected in the reaction of the commissural region to the inflammatory process.

## MATERIAL AND METHODS

This report is based on a study of 70 rheumatic hearts, together with 50 normal hearts. As in previous reports, the rheumatic material was segregated into six groups representing the various clinical courses that the rheumatic process may run as follows:

GROUP I. Active cases where death took place during the first attack (9 cases).

GROUP II. Active cases where one preceding attack occurred within 1 year of the fatal outcome (9 cases).

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- GROUP III. Active cases where one previous attack occurred at least 2 years previous to the fatal outcome (7 cases).
- GROUP IV. Active cases with a history of repeated attacks, death occurring during an acute recurrence (11 cases).
- GROUP V. Active cases where death was caused by decompensation without clinical evidence of a final recurrence. In some of these cases there was no previous history of rheumatic fever (25 cases).
- GROUP VI. Inactive cases of chronic valvular disease of the typical rheumatic variety (9 cases).

The specimens were fixed in 10 per cent neutralized formol-saline,\* standardized sections were taken according to the method of Gross, Antopol and Sacks,<sup>10</sup> and histological preparations were made using the technique employed by Gross and Ehrlich.<sup>1</sup> In a number of instances serial sections were made. In order to determine the optimum site and orientation of the sections for the study of these lesions vertical and horizontal serial sections were made through each of the aortic commissures in a number of rheumatic hearts. It appeared from these preliminary studies that microscopic changes were generally present in all the commissural regions and that these were more readily discernible in longitudinal cross sections (parallel with the long axis of the aorta). This section has the advantage of disclosing the aortic root, the aortic wedge (*i.e.* the wedge shaped termination of the aorta proper), the annulus extension of the aorta, the pericardial mantle and, occasionally, the aortic valve ring. For simplicity of approach the right-posterior commissure † of the aortic valve was chosen for study. This report will confine itself exclusively to this region.

\* Solution of formaldehyde U. S. P. 10 parts, 1 per cent sodium chloride solution 90 parts. This solution is rendered neutral with a weak alkali.

† For terminology of these commissures and other cardiac sites see Gross, Antopol and Sacks,<sup>10</sup> and Gross and Kugel.<sup>11</sup>

MICROSCOPIC APPEARANCE OF THE NORMAL RIGHT-POSTERIOR  
COMMISSURE

(50 Cases)

A description of this region must take into account the age period changes in the topographical and histological arrangement of the aortic root, the aortic wedge (the apical portion of the root that becomes continuous with the aortic annulus), the aortic annulus, the aortic ring and subvalvular angle, and the pericardial mantle.

The aortic root, by which is meant the terminal 2 cm. of the aorta proper just prior to its termination as a wedge shaped structure (on cross section), displays capillaries in its outer third from approximately the first year on. These are generally circular on cross section, very inconspicuous, and in normal roots are never surrounded by inflammatory cells. From the middle of the first decade on, there appears to be a moderate increase in these rather inconspicuous capillaries. From the fourth decade on, the capillaries occasionally appear as dilated sinusoids, and rare lymphocytes may be present in their vicinity. During the later decades (sixth decade on) the capillaries are not infrequently even more numerous. This may be due to sclerotic changes which are frequently present during these age periods.

The aortic wedge (Fig. 1) displays capillaries from about the middle of the first decade. These are more apt to be of the wide sinusoid variety. Muscular wall vessels are infrequent. From about the fourth decade on, these sinusoids often appear to be more conspicuous at the boundary between the aortic wedge and the annulus extension. The musculo-elastic structure of the aortic wedge is generally quite regular until about the fifth decade when a variable amount of irregularity appears, due to the occasional penetration of irregular capillaries from the adventitial layer. This moderate distortion of the aortic wedge architecture should not be confused with scarring.

The annulus is normally devoid of capillaries and blood vessels. No inflammatory cells are present. The only age period change worthy of note is the development of hyalinization and the deposition of lipoid material in the later decades.

As has been previously described,<sup>11</sup> the aortic valve ring rarely contains capillaries, inflammatory cells are not normally present,

and the ring spongiosa is generally sharply defined until the later decades of life, when a certain amount of scarring takes place. No reduplications are present in the subaortic angle, although elastification in this site occurs in the later decades.

The normal structure of the pericardium has already been described by Friedberg and Gross.<sup>7</sup> Worthy of note in connection with the present study is the fact that the pericardial collagen fibers adjoining the adventitial layer of the aortic root only infrequently show a moderate amount of condensation. The pericardial layer external to this zone (Fig. 1) contains delicate fat septa, a moderate number of capillaries and nerve trunks, and an intact lamina propria. Scattered lymphocytes are occasionally noted throughout this adipose layer.

#### MICROSCOPIC APPEARANCE OF THE RHEUMATIC RIGHT-POSTERIOR COMMISSURE IN GROUP I

##### *(9 Active Cases Where Death Took Place During the First Attack)*

In the description of the lesions present in this group and those subsequently to be discussed capillaries or cells will be considered as increased only when their incidence is greater than what one would expect to find normally in the corresponding age period.

Taken as a whole there was a distinct increase of capillaries in the aortic root in this group. These capillaries, surrounded by sparse lymphocytes, sometimes extended into the inner third of the aorta. Occasionally the aortic intima showed an inflamed reduplication (Gross<sup>4</sup>). The aortic wedge generally presented a marked increase in capillaries. These were almost invariably surrounded by inflammatory cells, chiefly lymphocytes, but occasionally also plasma cells and polymorphonuclear leukocytes. Incidentally, in referring to the increase in cells in this and other structures in all the groups under discussion it is to be noted that the lymphocyte is the most prominent cell and that plasma cells and polymorphonuclear leukocytes are generally present in considerably smaller numbers. The aortic wedge was moderately scarred (Fig. 2). This, however, was generally rather inconspicuous although it was occasionally quite severe. The aortic annulus extension as well as the ring annulus was frequently permeated with numerous capillaries surrounded by cells. These capillaries extended into the aortic ring and some-

times penetrated for a considerable distance down the annulus. The cells and capillaries in the aortic root, wedge, annulus and valve ring could be definitely traced as a contiguity process from lesions to be described in the pericardial mantle.

The rheumatic aortic valve ring lesions have been described in detail in a previous report.<sup>8</sup> Briefly, the most conspicuous changes found in this structure in the Group I cases were marked capillarization and inflammatory cell infiltration, slight scarring and inflamed subaortic angle reduplications.

Of considerable interest is the fact that in this group the pericardium almost invariably presented a dense collagenous zone of various thickness which merged imperceptibly with the aortic adventitia (Fig. 2). In about two-thirds of the cases there was present a moderate scattering of lymphocytes in this condensed zone. Less frequently, scattered lymphocytes were seen in the outer layers of the pericardium where, in addition, occasional thickenings of fat cell septa and capillary congestion were present. Almost invariably there could be observed lesions in the pericardial blood vessels. These vascular lesions were generally more conspicuous in the condensed collagenous zone referred to above and consisted of intimal fibrosis, intimal elastification, moderate hypertrophy and, on rare occasions, of intimal musculo-elastic hyperplastic changes.<sup>3</sup>

Summarizing these findings it appears that in Group I the commissural lesions consist of an increased capillarization of the aortic root, occasional inflamed reduplications of the aortic intima, somewhat more extensive capillarization and cellular infiltration of the aortic wedge with moderate scarring, permeation of the annulus extension and ring annulus by numerous capillaries and cells, active ring lesions, a zone of collagenous condensation in the pericardial mantle containing vascular lesions and lymphocytes, and moderate inflammatory changes in the other pericardial layers.

#### MICROSCOPIC APPEARANCE OF THE RHEUMATIC RIGHT-POSTERIOR COMMISSURE IN GROUP II

*(9 Active Cases Where One Preceding Attack Occurred Within 1 Year of the Fatal Outcome)*

As in the valve rings and leaflets, the aortic commissure in this group presented considerably more dramatic changes than in

Group I. Capillarization of the root and wedge was much more extensive, inflammatory cells (chiefly lymphocytes) were quite conspicuous and the annulus showed a considerable increase in distorted capillaries, sometimes in muscular wall vessels. As in the previous group, the vessels and inflammatory cells showed a contiguity process that extended from the pericardial mantle, through the aortic root wedge and annulus into the aortic ring. Aortic intimal reduplications were frequently present.

While scarring of the aortic wedge was not as pronounced as in the groups to be subsequently described, it was quite definite in the majority of cases. Occasionally an isolated island or islands of musculo-elastic wedge tissue were cut off from the remainder of this structure.

The most conspicuous changes found in the aortic valve ring in the Group II cases were marked inflammatory cell infiltration and vascularization, the latter consisting frequently of vessels showing intimal musculo-elastic hyperplastic changes, pronounced scarring and multiple, inflamed, vascularized subaortic angle reduplications, frequently with verrucous change.

The alterations in the pericardial mantle were quite characteristic. The zone of collagenous condensation was very wide and considerably inflamed. The outer layers of the pericardium frequently showed the following changes: thickening of the fat septa, frequently with increased fibrosis, conspicuous infiltration with lymphocytes and striking vascular changes occurring throughout the entire pericardial mantle, consisting chiefly of medial hypertrophy, intimal fibrosis and elastification, giant medial hypertrophy, intimal musculo-elastic hyperplastic changes and granular plugging of the vessels. Occasionally the surface of the pericardium presented a fresh fibrinous deposit.

Summarizing these findings it appears that in Group II the commissural lesions consist of marked capillarization and inflammatory cell infiltration of the aortic root, wedge, annulus, ring and pericardial mantle; a contiguity process can be traced from the pericardium to the aortic valve ring; muscular wall vessels occasionally penetrate the aortic root; the aortic wedge shows considerable scarring with isolation of musculo-elastic islands; intimal musculo-elastic hyperplastic vessels are found in the various lesions; the ring lesions are active and intimal musculo-elastic hyperplastic vascular

lesions and multiple subaortic reduplications are conspicuous; and the pericardial lesions are particularly active, consisting of a wide inflamed zone of collagenous condensation, widespread vascular lesions of various types and diffuse inflammatory cell infiltration.

### MICROSCOPIC APPEARANCE OF THE RHEUMATIC RIGHT-POSTERIOR COMMISSURE IN GROUP III

*(7 Active Cases Where One Previous Attack Occurred at Least 2 Years Previous to the Fatal Outcome)*

In this group the aortic root was also generally markedly capillarized and sometimes vascularized with muscular wall vessels. Cellular infiltrations were, however, usually quite mild. The aortic intima presented multiple, generally elastified reduplications in approximately half of the cases.

The aortic wedge was frequently considerably scarred with resulting severe distortion of the wedge architecture and, sometimes, nipping off of musculo-elastic islands (Fig. 3). The deformity of the wedge was occasionally so severe that a semblance of elastica whorling was produced. The bands of scar tissue often contained blood vessels which extended from the pericardium. These were frequently of the intimal musculo-elastic hyperplastic type. As in the first group, the wedge lesions were much more severe than those in the aortic root and the cellular infiltration was usually mild.

The annulus lesion consisted of a conspicuous permeation with capillaries and, sometimes, with muscular wall vessels. These lesions were associated with considerably more cellular exudate than that found in the aortic root or wedge. The annulus lesion was generally extensive and often assumed an appearance suggestive of collagen whorling (Fig. 3).

The ring lesions which were also not infrequently in distinct contiguity with the annulus and, through this structure, with the pericardium, consisted briefly of the following: an almost invariable but at times somewhat milder exudative lesion with capillarization and vascularization with muscular wall vessels, and the invariable presence of inflamed, generally multiple, vascularized subaortic reduplications.

In contrast to the previous group, cells were rather infrequent in the pericardium. The zone of collagenous condensation referred to



above was much less conspicuous and was frequently absent (Fig. 3). In about half the cases the fat septa showed moderate thickening. Blood vessel changes were frequently found in the pericardium and consisted generally of medial hypertrophy, intimal fibrosis and sometimes of intimal musculo-elastic hyperplastic changes.

Summarizing these findings it appears that in Group III the commissural lesions consist of marked capillarization and sometimes vascularization (with muscular wall vessels) of the aortic root, wedge and annulus, mild cellular exudate in these structures, pronounced scarring of the aortic wedge with isolation of musculo-elastic islands, distortion of wedge elastica and annulus collagen producing lesions that resemble whorling, contiguity extension of the vascular lesions from pericardium to ring, multiple elastified reduplications of the aortic intima, moderate activity of the ring with multiple, vascular inflamed reduplications, and somewhat milder pericardial lesions with either inconspicuous or no collagenous condensation zone formation.

#### MICROSCOPIC APPEARANCE OF THE RHEUMATIC RIGHT-POSTERIOR COMMISSURE IN GROUP IV

##### *(11 Active Cases With a History of Repeated Attacks, Death Occurring During an Acute Recurrence)*

In this group the aortic root also almost invariably presented increased capillaries which sometimes penetrated into the inner third of the aorta. Scatterings of lymphocytes around these vessels were rare. The aortic wedge was frequently richly vascularized. This vascularization differed from the previous three groups in that it generally consisted of thick muscular walled vessels. These also extended from the pericardium. Although inflammatory cells were infrequent in the aortic wedge they were somewhat more numerous than in the aortic root. Scarring of the aortic wedge was variable and was often quite severe. The intima of the aorta almost invariably showed multiple reduplications (Fig. 4). The annulus extension was usually permeated with distorted capillaries. At times these were infrequent. Cellularity was mild. The annulus lesion frequently extended for a considerable distance in all directions.

In brief, the ring lesions consisted of considerable vascularization, sometimes with intimal musculo-elastic hyperplastic changes but with very few inflammatory cells; at times only a few capillaries were found in the ring, and the subaortic angle almost invariably presented multiple elastified reduplications.

The zone of collagenous condensation in the pericardium was generally very broad and occasionally involved the entire thickness of the pericardial mantle (Fig. 4). Inflammatory cells were infrequent and when present were generally confined to the condensed pericardial zone. The fat septa were often thickened or congested. The pericardium almost invariably showed vascular changes consisting of medial hypertrophy, intimal fibrosis and occasionally giant medial hypertrophy or intimal musculo-elastic hyperplastic changes. These vessel changes were generally confined to the zone of collagenous condensation.

Summarizing these findings it appears that in Group IV the commissural lesions consist of increased capillarization of the aortic root, conspicuous vascularization of the aortic wedge with thick muscular walled vessels and a variable amount of scarring, permeation of the annulus with distorted capillaries, mild inflammatory cell infiltration of these lesions, multiple elastified reduplications of the aortic intima and subaortic angle, mild but vascularized ring lesions, and broad collagenous condensation zones in the pericardium, with mild cellularity but fairly advanced vascular changes.

#### MICROSCOPIC APPEARANCE OF THE RHEUMATIC RIGHT-POSTERIOR COMMISSURE IN GROUP V

*(25 Active Cases Where Death Was Caused by Decompensation Without Clinical Evidence of a Final Recurrence. In Some of these Cases There Was No Previous History of Rheumatic Fever)*

Group V represented a considerably milder clinical course of rheumatic fever and, as with lesions previously described in other cardiac sites, was associated with much less alteration in the aortic commissures. Thus, the aortic root generally showed fewer capillaries than in the preceding groups, although occasionally capillarization was quite extensive. Lymphocytes, while generally present, were extremely inconspicuous. In approximately one-third of the

cases the aortic intima presented flat elastified reduplications. Capillarization of the aortic wedge was generally greater than that of the root. These capillaries were often associated with a variable amount of scarring. In about one-fourth of the cases the scarring was severe and resulted in elastica whorling (Fig. 5); in approximately one-fourth of the cases no scarring was present. No increase in inflammatory cells was noted in the aortic wedge in about half of the cases. When present they were sparse and somewhat more conspicuous than in the aortic root.

The annulus was generally much freer from inflammatory changes than in previous groups. In approximately two-thirds of the cases the annulus presented capillaries, occasionally muscular walled vessels. In a few cases the vessels were confined to the annulus-wedge boundary. Cells were infrequent. Although generally mild, the annulus lesion was sometimes quite widespread and associated with collagen whorling.

The ring lesions were considerably subdued in this group and consisted generally of thick walled muscular vessels or distorted capillaries, advanced scarring, sparse cellularity, and multiple elastified vascularized reduplications in about half the cases.

In this group the pericardium differed from that seen in the other groups in that there was found no zone of collagenous condensation in the majority of cases (Fig. 5). When present it was generally narrow. Lesions of the pericardial vessels were found less frequently than in the previous groups. When present they occurred chiefly in the narrow zone of collagenous condensation and consisted of medial hypertrophy, intimal elastification and occasionally giant medial hypertrophy. In about half of the cases no vascular lesions were seen. Cellularity was most inconspicuous and when present the lymphocytes were scattered between the fat cells rather than in the collagenous zone of condensation.

Summarizing these findings it appears that in Group V the aortic root and wedge show fewer capillaries than in the preceding groups; flat aortic intimal reduplications occur only in one-third of the cases; scarring of the aortic wedge is variable but generally less conspicuous than previously described; inflammatory cell infiltration is very mild; the annulus lesions are similarly less involved but there is a tendency for vessels to be more prominent at the annulus wedge boundary; ring lesions contain thick walled vessels and distorted

capillaries, but few inflammatory cells; the pericardium rarely shows a collagenous condensation zone; and vascular lesions and cell infiltrations are rather inconspicuous.

#### MICROSCOPIC APPEARANCE OF THE RHEUMATIC RIGHT-POSTERIOR COMMISSURE IN GROUP VI

##### *(9 Inactive Cases of Chronic Valvular Disease of the Typical Rheumatic Variety)*

The only consistent alteration in this group was a moderate increase in capillaries in the aortic root with mild scatterings of lymphocytes. No reduplications were noted in the intima. The aortic wedge showed slight scarring in about half the cases and capillaries were somewhat more numerous at this site. In 1 case the wedge was penetrated by muscular walled vessels. When capillaries were present in the wedge they were generally distorted. Inflammatory cells were infrequent. The annulus showed capillarization in only 2 cases but no inflammatory cells were present. When lime was present near the aortic ring there occurred a considerable increase in blood vessels, capillaries and cells. Otherwise the valve rings were generally severely scarred and almost completely devoid of inflammatory cells. When vascularization occurred it consisted of capillaries, hyaline arterioles and hypertrophied vessels. In half the cases multiple elastified subaortic reduplications were present.

One of the most characteristic features of this group was the extreme paucity of pericardial lesions behind the commissure. Thus, zones of collagenous condensation were infrequent and, when present, were narrow and irregular; lymphocytes were noted in only 2 cases and in these they tended to be concentrated in the outer layers of the pericardium; and vascular lesions were not observed. In most cases the septa between the fat cells were somewhat thickened.

Summarizing these findings it appears that in Group VI the aortic root presents a moderate increase in capillaries but no intimal reduplications; the aortic wedge shows slight scarring in about half the cases, and a somewhat greater increase in capillaries; the annulus is relatively free of inflammatory lesions; inflammatory cells are infrequent in these sites; the ring lesions are practically completely inactive, about half the cases showing only vestiges of previous in-

flammation in the form of severe scarring, capillarization, thickened arterioles and muscular vessels, and subaortic multiple elastified reduplications; and the pericardial mantle is relatively free of inflammatory alterations.

## DISCUSSION

A brief review of the inflammatory changes found in the aortic commissures of the six groups herein described reveals a remarkable parallelism with the clinical course of the rheumatic disease, and thus agrees closely with the findings previously described in other cardiac sites. Commissural lesions were most conspicuous and active in the first four groups, diminished strikingly in activity in the fifth group, and existed only as vestiges in the sixth.

The first group showed considerable capillarization of the aortic root and its extensions, fairly marked inflammatory cell infiltration, relatively little scarring, and inflammatory cells in the pericardium with a zone of collagenous condensation and vascular changes. The second group differed in the somewhat more extensive inflammatory changes, the appearance of muscular walled and intimal musculo-elastic hyperplastic vessels in various sites, greater scarring of the aortic wedge with nipping off of musculo-elastic islands, extraordinarily active ring lesions, and intensely inflamed pericardium with a wide collagenous condensation zone, and vascular lesions in great variety. The third group was somewhat similar to the second except that the aortic intima presented multiple reduplications, inflammatory cell infiltration was less severe, the pericardial collagenous condensation zone was usually narrower or absent, and the pericardial vascular lesions not so varied. The fourth group also showed a mild grade of exudative phenomena, the aortic wedge was frequently vascularized with muscular wall vessels and the pericardial collagenous condensation zone was very broad. In Group V inflammatory cells were quite sparse, the aortic root intimal reduplications were single, flat and less frequently present, wedge lesions were less severe, the annulus lesions were less frequent and the pericardial lesions were quite mild. In Group VI activity was practically nil; when present the lesions were chiefly confined to the aortic wedge and the pericardium was relatively free of alterations but inconspicuous vestiges of inflammatory lesions could sometimes be found in the root, wedge and ring.

The topographical features of some of these lesions, together with the constancy of the findings, clearly indicate the pathogenesis of the commissural lesion. In a previous report Gross and Friedberg<sup>8</sup> described several avenues through which the valve rings may be affected. It was shown that one of these is by way of the adjacent pericardial mantle which is in intimate relation to the valve rings in the region of the great vessel roots.<sup>11</sup> In a recent report by Gross<sup>4</sup> it was shown that the great vessel roots are frequently the seat of inflammatory disturbances during the course of a rheumatic process. It was indicated in this report that the pericardium may play a not insignificant rôle in the initiation and continuation of inflammatory phenomena at this site. These observations receive further confirmation through the findings described in this report. As mentioned above, in all the active rheumatic groups the pericardial mantle of the aortic valve commissure showed pronounced inflammatory changes. These consisted of zones of collagenous condensation representing organization of inflammatory tissue, inflammatory cells varying in amount with the acuity of the process, conspicuous changes in the blood vessels of the pericardium, and penetration of these vessels into the aortic root, aortic wedge and annulus. From the latter site the penetration could be traced directly to the aortic ring where the inflammatory process generally appeared to be most active. Furthermore, there was present a large graded series of lesions which presented all stages in the penetration of granulation tissue from the pericardium inward toward the ring. Thus, although it cannot be denied that the initial rheumatic infection may reach the valve ring from other sources, such, for example, as the intervalvular fibrosa or the underlying myocardium, and spread in retrograde fashion to the adjacent pericardium, the mechanism of granulation tissue penetration into the rheumatic aortic ring is undoubtedly chiefly by contiguity from the latter site. Granted, then, infection in the aortic valve ring, with coincidental or subsequent granulation tissue permeation, this spreads distally into the valve leaflets and, because of their close apposition at the commissural region, leads to agglutination of their edematous and inflamed ventricularis mantles. Apparently the constant apposition of these leaflets and, possibly, the additional trauma imposed on them by their systolic and diastolic movements, lead to increased inflammatory phenomena with agglutination and organization.

It is of considerable interest that the clinical course of the disease is accurately depicted by the qualitative and quantitative differences in the commissural lesions described above. The presence of these lesions in very high incidence constitutes another group of stigmata by which extinct rheumatic fever may be recognized, for it has been shown that even in the completely indolent Group VI evidence of such past infection can sometimes be observed.

The lesions of the aortic root have already been described in considerable detail elsewhere.<sup>4</sup> In the present studies it is shown that scarring of the aortic wedge with distortion of the normal architecture is of frequent occurrence in rheumatic fever and that in the more active groups the distortion may become quite severe. That it never reaches the extreme grade of destruction seen in syphilis (Fig. 6) differentiates the rheumatic lesion very easily from the former and also affords an explanation for the absence of the characteristic commissural separation seen so frequently in aortic root syphilis. On the other hand, it is not inconceivable that a marked grade of destruction in the aortic wedge produced early in life may so completely distort the normal topography of this structure that there may be produced a picture suggestive of faulty inversion such as that described by Lewis and Grant<sup>12</sup> in the congenital bicuspid aortic valve. In this connection it is of considerable interest that elastica whorling of the wedge apex and collagen whorling of the annulus were not infrequently encountered in the severe lesions. These have also been considered indicative of a congenital lesion by the above mentioned authors. In the light of these findings, therefore, it seemed advisable again to study the pathogenesis of the so-called congenital bicuspid aortic valve from the point of view of the possible rôle played by inflammation, chiefly of the rheumatic variety, in the production of this lesion. This matter forms the basis of a separate report which has been published elsewhere.<sup>13</sup>

### SUMMARY AND CONCLUSIONS

There has been described in this report the findings in 70 cases of rheumatic fever segregated into six groups according to the clinical course taken by the disease. It is shown that a number of inflammatory changes are found in the aortic root, wedge, annulus, ring, sub-aortic angle and pericardial mantle which are characteristic of rheu-

matic fever and, to some extent, reflect the clinical course of the disease. Even when healing takes place the histological characteristics of the commissural lesion afford additional stigmata which are of value in discerning a past rheumatic process. A discussion is given of the pathogenesis of this lesion from which it appears that even though the original infection may reach the aortic ring through several routes, in most instances the inflammatory granulation tissue passes from the pericardial mantle through the aortic root, wedge and annulus to reach the aortic rings. The latter show a much more flagrant inflammatory process which spreads into the valve leaflets and, probably with the additional factor of trauma caused by the systolic and diastolic movements of the cusps, eventually leads to their agglutination. The possible significance of these findings in relation to the pathogenesis of the so-called congenital bicuspid aortic valve is indicated. A description is also given of the histological and topographical changes taking place during the different age periods in the normal aortic commissural region.

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## DESCRIPTION OF PLATES

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### PLATE 60

FIG. 1. Longitudinal cross section through the right-posterior aortic valve commissure from a normal control case. Age 50 years. Low power. Weigert's elastic and Van Gieson's connective tissue stain.

A = aortic wedge; B = sinusoidal capillary; C = ring annulus; D = pericardium.

FIG. 2. Longitudinal cross section through the right-posterior aortic valve commissure from a rheumatic case classified as Group I. Age 9 years. Low power. Weigert's elastic and Van Gieson's connective tissue stain.

A = aortic wedge; B = scarred apex of aortic wedge with penetrating capillaries; C = ring annulus containing few capillaries; D = capillarized annulus extension. Note penetration of capillaries from pericardium. E = capillarized aortic ring; F = subaortic reduplication; G = capillarized inflamed reduplication in aortic sinus pocket; H = collagenous condensation zone in pericardium; J = blood vessel showing intimal fibrosis; K = inflamed adipose tissue.

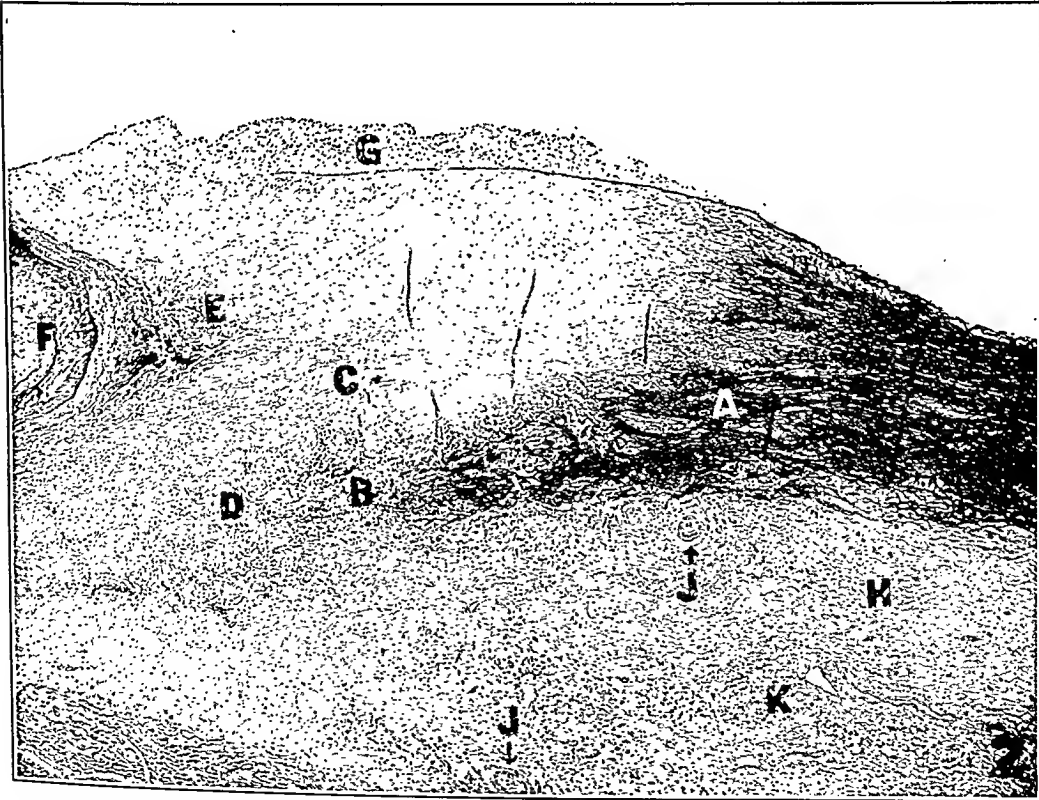
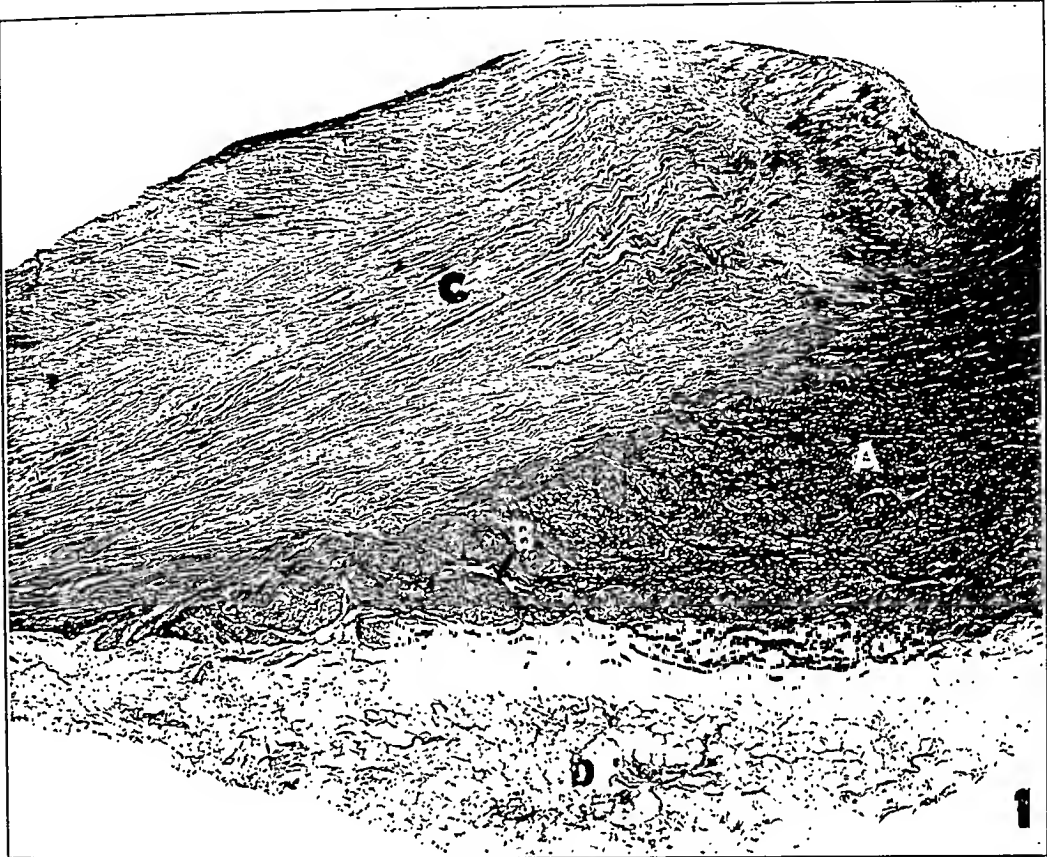


PLATE 61

FIG. 3. Longitudinal cross section through the right-posterior aortic valve commissure from a rheumatic case classified as Group III. Age 19 years. Low power. Weigert's elastic and Van Gieson's connective tissue stain.

A = aortic wedge; B = penetrating muscular wall vessel with scar tissue nipping off musculo-elastic island; C = almost complete obliteration of wedge apex. Note numerous blood vessels. D = vascularized ring annulus; E = vascularized ring; F = vascularized subaortic reduplications; G = pericardium containing altered blood vessels. Note absence of collagenous condensation zone. H = blood vessel showing intimal musculo-elastic hyperplastic changes; J = posterior aortic leaflet.

FIG. 4. Longitudinal cross section through the right-posterior aortic valve commissure from a rheumatic case classified as Group IV. Age 20 years. Low power. Weigert's elastic and Van Gieson's connective tissue stain.

A = aortic wedge; B = capillarization and scarring of aortic root; C = multiple aortic intimal reduplications; D = capillarized ring annulus; E = collagenous condensation zone in pericardium; F = altered blood vessels.

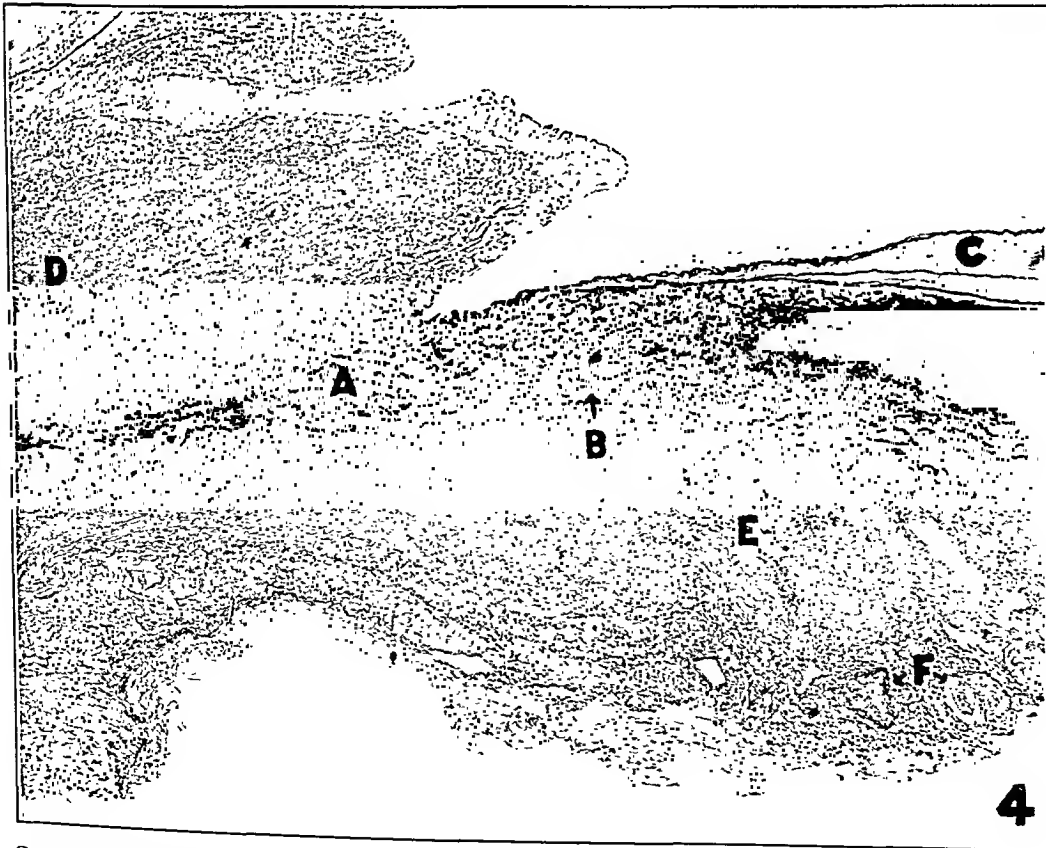
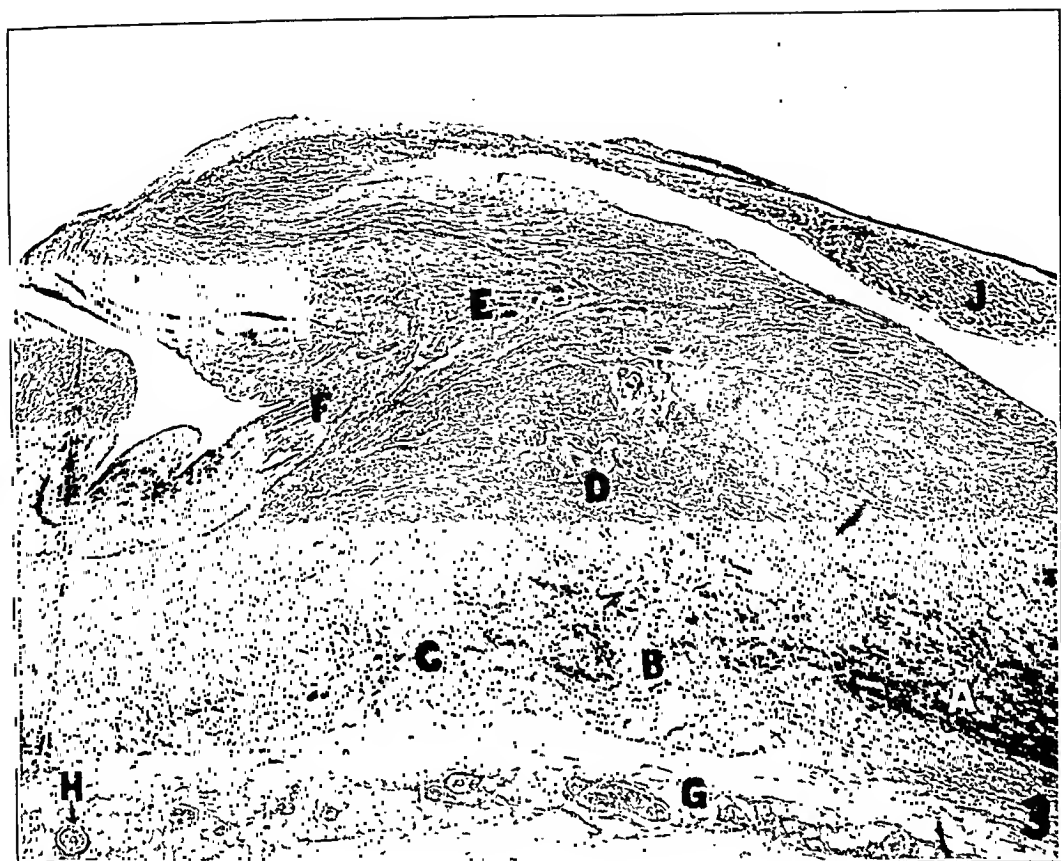


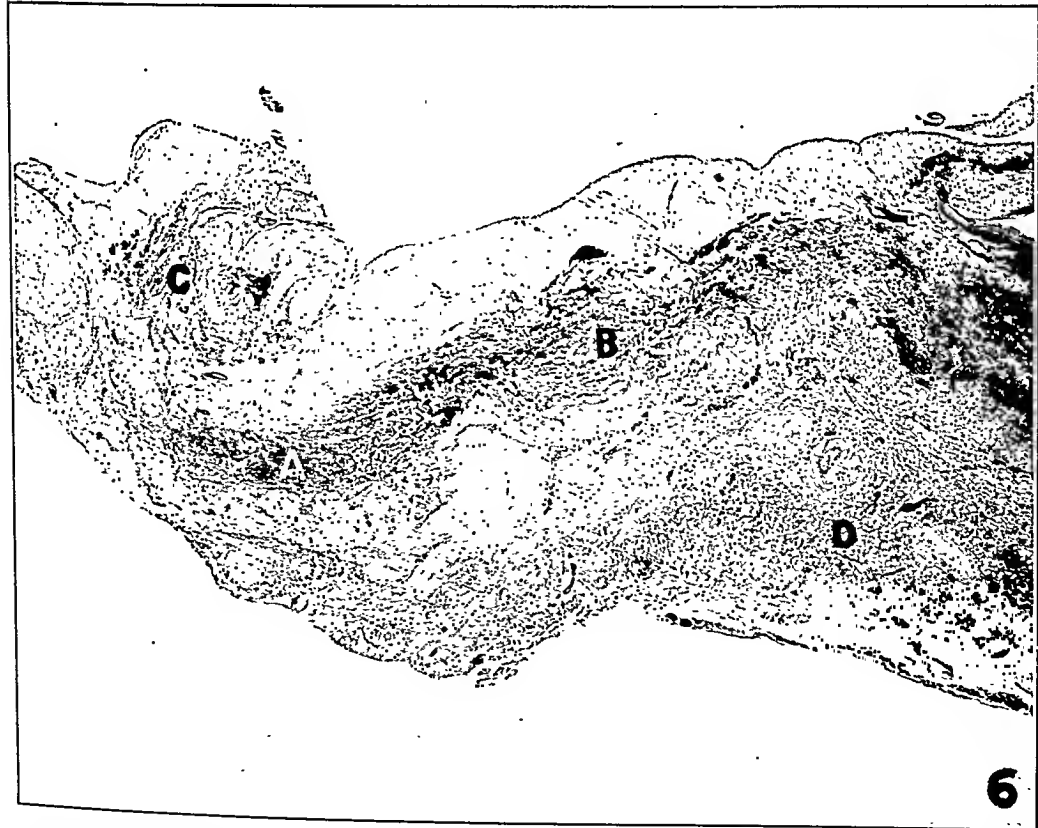
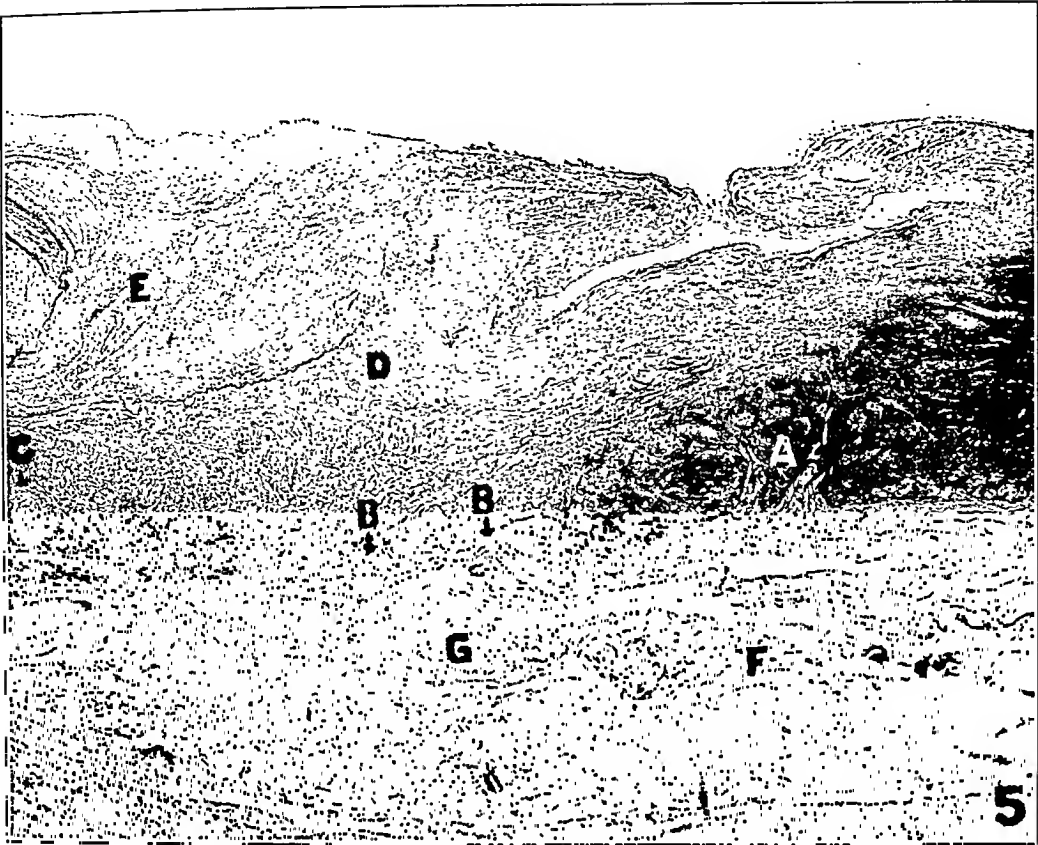
PLATE 62

FIG. 5. Longitudinal cross section through the right-posterior aortic valve commissure from a rheumatic case classified as Group V. Age 29 years. Low power. Weigert's elastic and Van Gieson's connective tissue stain.

A = severely distorted aortic wedge due to penetrating blood vessels and scar tissue; B = scar tissue nipping off musculo-elastic islands; C = muscular walled vessels in annulus extension; D = scarred ring; E = vascularized subaortic reduplications; F = pericardium containing (G) enormously hypertrophied vessels with intimal fibrosis. Note absence of collagenous condensation zone.

FIG. 6. Longitudinal cross section through the right-posterior aortic valve commissure from a case with aortic root syphilis. Age 37 years. Low power. Weigert's elastic and Van Gieson's connective tissue stain.

A = distorted aortic wedge; B = severe scarring of aortic wedge and root producing almost complete destruction of elastic and muscular tissue; C = severe ring lesion; D = pericardium showing intense inflammation with severe vascular changes.





# THE MINERAL CONTENT OF VARIOUS CEREBRAL LESIONS AS DEMONSTRATED BY THE MICROINCINERATION METHOD \*

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## INTRODUCTION

Microincineration was first applied to botanical studies by Raspail<sup>1</sup> in 1833. Liesegang<sup>2</sup> in 1910 studied by means of this method the mineral residue of blood in the capillaries of embryonic brain, lens, retina and optic nerve of fish. Herrera<sup>3</sup> (1913), Naumann<sup>4</sup> (1915), Prenant<sup>5</sup> (1919), and Molisch<sup>6, 7</sup> (1920-1921) made use of it in various botanical and zoological problems. In 1923 Policard<sup>8</sup> devised newer and more detailed methods of procedure and observation, and together with co-workers introduced the method into human histopathology.<sup>9-13</sup> Later Scott<sup>14-16</sup> revised and improved the method, especially by adding darkfield to Policard's observations in oblique light.

With this method the normal anatomy and pathology of many body tissues of man, vertebrates and invertebrates were studied and many fundamentally new observations were made. Clear recognition of silica (Herrera,<sup>17</sup> Lanzoni<sup>18</sup>) enabled Policard and Doubrow,<sup>19</sup> and later Lecloux,<sup>20</sup> to demonstrate associated silicosis in anthracosis of the lungs and thereby to interpret it in a new light. Policard and Pillet<sup>21</sup> discovered calcium in the liquid contained in the intracranial diverticula of the *saccus endolymphaticus* in frogs, and Baginski<sup>22</sup> demonstrated the calcium content of cartilage, also hyaline cartilage, which had not been demonstrable by staining methods. Kooyman<sup>23</sup> studied the minerals in the skin. Policard, Noël and Pillet,<sup>24</sup> Okkels,<sup>25, 26</sup> and others, studied changes in the

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mineral structure of organs produced by metabolic changes: after a carbohydrate diet mineral ash increases in the peripheral parts of liver lobules; in experimental calcium poisoning this mineral is deposited in the convoluted tubules and papillae of the kidney; and injected iron salts are deposited in the liver, spleen and kidneys in inverse order of their solubility.

The important question whether minerals in tissues, as demonstrated by microincineration, actually correspond in their arrangement to vital structures or not was successfully answered by Scott,<sup>27</sup> who proved their identity with the materials revealed by ultraviolet light photomicrography of living cells (Köhler,<sup>28</sup> Lucas,<sup>29</sup> Wyckoff and Ter Louw,<sup>30</sup> Lucas and Stark,<sup>31</sup> and Wyckoff, Ebeling and Ter Louw<sup>32</sup>). Scott also refers to the fact that for nuclei this had already been demonstrated by Policard.<sup>33</sup> This has also been confirmed by the studies of Gage, Day and Starrett.<sup>34</sup>

More difficult is the question of identification of the minerals observed. With certainty only three mineral elements can be identified, namely iron, calcium and silica. Policard,<sup>35</sup> in one of his first papers on the subject, found that by microincineration all iron compounds, including organic compounds — the so-called “masked iron” — are transformed into iron oxide ( $\text{Fe}_2\text{O}_3$ ), which stands out by its light yellow to dark red color. Where only a slight amount is present it is light yellow, as in fluid blood in vessels; where it is abundant the color is vivid red, as in phagocytic cells or in old hemorrhages. Scott<sup>15</sup> and others confirmed Policard's finding. Scott points to an important source of error in interpretation: in darkfield illumination carbon particles remaining in incompletely incinerated preparations also give a yellowish to red color; however, these carbon particles when viewed with transmitted light will appear black, while only iron oxide retains its color. Therefore it is necessary, before interpreting any colored particles to be iron, to ascertain by transmitted light whether or not the preparation has been completely incinerated. Microincineration, applied with this caution, is superior in accuracy of demonstrating iron in tissues to any known staining reaction (Scheid,<sup>36</sup> Marza, Marza and Chiosa<sup>37</sup>), even to Macallum's<sup>38-40</sup> methods.

Silica is present as a white, doubly refractive, crystalline mineral insoluble in water. Scott,<sup>15</sup> however, points to the fact that in all probability it is present as a silicocalcareous compound, and

therefore quantitatively its actual amount can easily be overestimated.

Calcium is present as a bright, white, monorefractive ash, which is practically insoluble in water (Schultz-Brauns<sup>41</sup>), although a small quantity of it may go into solution (Sheldon and Ramage,<sup>42</sup> Scott<sup>16</sup>). It may, furthermore, be ascertained by the gypsum or the oxalic acid reaction (Ostertag<sup>43</sup>). For interpretation of the distribution of calcium salts in tissues microincineration was most revolutionary and revealing. In many tissues (*i.e.* blood vessels, placenta) the usual staining reactions for calcium (Röhl-von Kóssa-hematoxylin) show only a relatively small part of the total amount of insoluble calcium salts, as demonstrated by microincineration (Schultz-Brauns and Schoenholz,<sup>44</sup> Schoenholz,<sup>45</sup> Zinkant<sup>46</sup>). In other instances, however, microincineration reveals the absence of mineral where the staining reactions seem to indicate the presence of calcium. According to Scott<sup>15</sup> the usual histochemical tests for calcium reveal it in large quantities in and about the parietal cells of gastric glands, yet microincineration, which offers but slight chance of any appreciable alteration in the location of minerals in cells and tissues, shows that little, if any, salt is present in these cells except in their nuclei.

The recognition of magnesium in microincinerated slides is unsatisfactory, especially in the presence of calcium (Scott<sup>15</sup>). Although attempts have been made to identify sodium and potassium (Jacobi and Keuscher,<sup>47</sup> Policard and Pillet<sup>48</sup>) their recognition is still unsatisfactory in spite of the fact that much less of these elements is volatilized by the heat of microincineration (Sheldon and Ramage,<sup>42</sup> Scott<sup>16</sup>) than previously assumed. According to Schultz-Brauns<sup>41</sup> the ashes of sodium, potassium and magnesium can be differentiated from the ash of calcium by their bluish hue, but although this may give practically useful clues it should be remembered that transmitted structural blue is due to smallness of quantity rather than to any inherent quality (Mason,<sup>49</sup> Scott<sup>16</sup>).

The microincineration method can be used only for estimation of the relative quantity of mineral ashes. It should be borne in mind that microincineration is a microscopic method, and still more specifically an ultramicroscopic method. Attempts to force quantitative chemical information (Schultz-Brauns,<sup>50</sup> Wulf,<sup>51</sup> Marza and Chiosa<sup>52</sup>) were doomed to failure. Microincineration at present

gives accurate information only as to the location of the total heat-resistant minerals and specifically of iron, silica and calcium in cells and tissues, lacking in other histochemical procedures; or, as Scott <sup>15</sup> concludes, "Observation of incinerated preparations gives an exact conception of the mineral structure of protoplasm." Finer analyses will have to be made by spectroscopic and chemical examination (Gerlach and Schweitzer,<sup>53</sup> Gerlach and Gerlach,<sup>54, 55</sup> Gerlach,<sup>56</sup> Policard,<sup>57</sup> Scott and Williams <sup>58</sup>).

Data on the nervous system, obtained by the microincineration method, are still scarce. Scott <sup>15</sup> studied the localization of mineral salts in the normal nervous system of mammalia. Of pathological lesions of the nervous system the best known as to mineral structure are inclusion bodies. Covell and Danks <sup>59</sup> found that Negri bodies in rabies contained a rather compact ash residue; intranuclear inclusions from the cerebral cortex of rabbits in herpes (Rector and Rector <sup>60</sup>), however, contain mineral only in their early stages and lose it later. In contrast to these the intranuclear inclusions in the submaxillary glands of guinea pigs (Cole and Kuttner,<sup>61</sup> Scott <sup>62</sup>), according to Scott,<sup>63</sup> and the intranuclear inclusions in yellow fever, according to Cowdry,<sup>64</sup> do not contain mineral. The inclusion bodies in fowl-pox were found to contain mineral by Danks.<sup>65</sup>

Scheid <sup>66</sup> described loss of mineral residue in granular cells of the cerebellum during diabetic coma. He also described the mineral structure of perivascular hemorrhages in the brain. Wulf <sup>51</sup> described iron in the vessel wall and in perivascular scavenger cells in a case of old perivascular hemorrhage. In an organized thrombus from a vessel of the human pons he described the net-like ash structure of the thrombus and iron in the wall of the thrombosed vessel. He emphasized that the pseudocalcium of vessel walls does not leave ash residue. He confirmed Scheid's finding of disappearance of the ash in the granular layer of the cerebellum in 2 cases of coma diabeticum, but points out, however, that in other of his cases (uremia, arteriosclerosis) the granular layer was poor in ash. Patton <sup>67</sup> studied the cell changes in experimental poliomyelitis and found that after a transitory stage of increase of minerals the diseased anterior horn cells became demineralized.

These findings seemed significant enough to warrant undertaking a systematic study of various cerebral lesions on a larger series of material.

## METHODS AND MATERIAL

In our studies we followed Scott's<sup>15, 16</sup> modification of the method and the general technical rules laid down by him.\*

The microincineration technique consists in exposing sections of tissue fixed in absolute alcohol and formalin,† embedded in paraffin,§ and cut at  $4\ \mu$ , to temperatures gradually rising to  $650^{\circ}\text{C}$ . By this procedure the organic components of the section are burnt out and only the mineral ash, or heat resistant mineral constituents of the tissue, remain on the slide. At low power the preparations are viewed with oblique transillumination, at high power by dark-field illumination. By this method the tissue minerals that are not volatilized or vaporized at temperatures up to  $650^{\circ}\text{C}$ . are demonstrated. These are the *heat resistant mineral ashes*, which make up an important portion of the mineral constituents of mammalian and human tissues.

Neighboring sections of all incinerated sections were cut and stained with one of the usual stains (cresyl violet, hematoxylin-eosin, Masson's trichrome stain, and some with a modification of Bielschowsky's silver impregnation for paraffin sections).

\* We are also obliged to Dr. Scott for having a furnace constructed in his laboratory and sent to us, which we used for most of our work.

In the latter part of our work we also used furnaces which are now manufactured by the A. S. Aloe Company in St. Louis, according to Dr. Scott's directions.

† From tissue fixed in 95 per cent alcohol satisfactory preparations can be obtained. Tissue fixed in 10 per cent formalin also gives comparable, though less brilliant pictures, if the blocks have not been left in formalin for more than 2 to 3 weeks. If possible, Scott's original fixative (absolute alcohol 9 parts, 40 per cent formalin 1 part) should always be chosen as it is superior to any other fixative in the brilliancy and completeness of the ash picture, and in avoiding any irregular shrinkage.

§ In a few cases additional frozen sections of 10 to  $16\ \mu$  from the same material (fresh and fixed) were incinerated. This procedure, though the pictures obtained corresponded essentially to those obtained from paraffin embedded material, at least at low power, has two distinct disadvantages: (1) a greater tendency to incomplete incineration of the tissue; and (2) lack of cytological detail. Both these disadvantages are due probably to the necessarily greater thickness of frozen sections. The results, however, were somewhat more satisfactory when frozen sections, before incineration, were passed through graded alcohols and ether.

From thin celloidin sections, 4 to  $8\ \mu$ , quite satisfactory pictures may be obtained. It is necessary, however, to dissolve the celloidin completely in alcohol and ether before incineration.

The most satisfactory method, however, is the use of paraffin sections cut at  $4\ \mu$ . All the descriptions and conclusions in our study are based on such sections and with exceptions of Figure 34 the illustrations are taken from them.

Our material consisted of brains from the following 40 human cases: \*

Normal adult (death from lobar pneumonia) . . . . .	2
Newborn premature . . . . .	2
Cerebral arteriosclerosis . . . . .	9
Senile and presenile atrophy (dementia)	
Alzheimer's disease . . . . .	4
Pick's disease . . . . .	2
Syphilitic meningoencephalitis and dementia paralytica . . . . .	4
Purulent meningitis (pneumococcus) with purulent meningoencephalitis . .	1
Chronic alcoholism (Korsakoff's psychosis and delirium tremens) . . . . .	3
Multiple sclerosis . . . . .	2
Diabetic coma . . . . .	2
Symptomatic psychosis	
in cardiorenal disease . . . . .	1
in pneumoconiosis . . . . .	1
Traumatic laceration of brain . . . . .	1
Manic depressive psychosis	
complicated by emaciation . . . . .	1
complicated by pulmonary and intestinal tuberculosis . . . . .	1
Schizophrenia	
complicated by emaciation . . . . .	1
complicated by pulmonary and intestinal tuberculosis . . . . .	1
Brain tumor	
Glioma (astroblastoma) . . . . .	1
Metastases from carcinoma of bronchial epithelium with squamous metaplasia . . . . .	1

From these cases the following cortical areas were examined: central region (areas 4, 6, 3, 2, 1), area frontalis granularis (10), area calcarina (17, including parts of 18), Ammon's horn, and area entorhinalis. In addition to these cortical areas we examined blocks from the striatum, thalamus and hypothalamus, cerebellar cortex and medulla oblongata. In some cases we examined also sections from the spinal cord.

In addition to this human material we have been collecting several series of experimental animal material with normal control tissue from the same animals, including brains and body tissues of dogs, cats, rabbits, guinea pigs and pigeons.

\* Nine of these cases were obtained from the autopsy material of the Mallory Institute of Pathology and the Neuropathological Laboratory of the Neurological Unit of the Boston City Hospital and 2 cases from the Worcester State Hospital, through the courtesy of Drs. F. Parker, Jr., and T. J. Putnam, and Drs. J. M. Looney and W. Freeman.

## MINERAL STRUCTURE OF THE CEREBRAL CORTEX OF THE NEWBORN

The cerebral cortex of the human newborn is rich in mineral ash. Sections show a great amount of mineral in the nuclei of ganglion cells and glial elements of all cortical layers (Fig. 1). The cytoplasm of these cells, however, contains none or very little mineral ash. A slight yellowish hue here and there in the nuclear mineral ash is indicative of the presence of iron; however it is far surpassed in relative amount by calcium and probably also, although in lesser degree, by some water soluble minerals. These findings show that minerals are inborn in the cerebral cortex of man. These minerals however are, in contrast to the arrangement in adults, accumulated in the nuclei of ganglion cells. Also, other actively growing tissues show nuclear mineral deposits, as can be seen from the literature and from our studies.

This finding bears upon an important point, namely the biological significance of tissue minerals. It has been a common belief that minerals in the tissue appear especially where necrosis has occurred. Even in the beginning of the work on microincineration some authors still clung to this concept. Schultz-Brauns<sup>41</sup> considered deposits of calcium as occurring either in tissues and cells which are necrotic, or in cells with decreased function and metabolism, in the sense of Freudenberg and György.<sup>68</sup> In another paper Schultz-Brauns and Schoenholz,<sup>44</sup> although stressing the presence of mineral, and especially of calcium, in living tissue, *i.e.* in living placental epithelium and in migrating histiocytes of the chorion, still consider it as indicative of cell damage. They consider even the migrating histiocytes that contain calcium to be damaged. This interpretation is made in spite of the findings of Wehefritz<sup>69</sup> and Schoenig,<sup>70</sup> who from clinical, chemical and physiological points of view interpreted the presence of the larger quantities of calcium in early growing placentas as indicating a physiological storage of calcium. They considered calcium in the necrotic areas of placental infarcts pathological or degenerative in nature. In a later paper Schultz-Brauns<sup>71</sup> claims that calcium deposits in cortical ganglion cells of the cerebrum are pathological.

Scott<sup>63</sup> changed this concept by clearly and irrefutably demonstrating that minerals, including calcium, are an inherent part of

the nucleus of the actively growing and dividing cell, and in their arrangement correspond to the nuclear chromatin, especially in cells in mitosis (Scott,<sup>14, 72</sup> Funaoka and Ogata<sup>73</sup>). Recently Scott<sup>74</sup> confirmed this finding in tissue treated by the Gersh-Bensley method. Policard<sup>75</sup> found mineral, most of it calcium and magnesium, in the nuclei, and less of it in the cytoplasm of macrophages from tissue cultures. Horning and Scott,<sup>76</sup> studying tissue cultures of mesodermal cells, found no visible gradation in the mineral content between the elements nearer the original implant and those that migrated the farthest into the liquid medium, thus forming the growing edge of the culture. However, while tissue cultures contain a fair amount of calcium oxide and sodium they are practically free of iron. Clasmotocytes and other ameboid elements are the most outstanding in tissue cultures, owing to the enormous concentration of mineral salts (calcium) within the main body of the cell and in their pseudopodia. Policard<sup>77</sup> found the nuclei of the seminal cells and the spermatozoa in man and in rats rich in minerals. In the spermatozoa he found calcium, as well as traces of iron, the latter, however, in the anterior part of the head only. Marza, Marza and Chiosa,<sup>37</sup> and later Baginski,<sup>78</sup> found minerals but no iron in the nucleus of the ovum (oocytes and de Graaf's follicles). Iron in the ovary was found in two forms: (1) inorganic in the interstitial cells; and (2) organic in the vitellus of the ovum during the period of rapid growth. Horning and Scott<sup>79</sup> demonstrated the inorganic structure of the growing organism by studying the distribution and changes in inorganic salts during embryonic development of the chick. Embryonic development is inorganically expressed by an increase in certain mineral salts and a decrease in others. During growth of the brain there is a gradual increase in calcium content, while iron salts decrease. The growing cells, and especially their nuclei, were found to be rich in mineral residue.

MINERALS IN GANGLION CELLS OF THE CEREBRAL CORTEX,  
CEREBELLAR CORTEX AND SPINAL CORD OF THE NORMAL  
ADULT WITH A DISCUSSION OF THEIR PATHOLOGICAL  
ALTERATIONS

In incinerated preparations of human cerebral cortex the layers of cortical ganglion cells stand out at low power like the negative of a Nissl preparation (Fig. 3), however, with one difference. This

consists in the fact that in the cerebral cortex granular cells contain much less mineral than large and small pyramidal cells. Therefore, the granular layers stand out as less mineralized than the pyramidal. The glial ground net stands out as a fine network containing slight traces of mineral (Fig. 9). The nuclei of glial cells show mineral residue which is most compact in the nuclei of the oligodendroglia cells of normal brain.

The mineral in cortical ganglion cells of the pyramidal type in the adult is restricted to the cytoplasm and nucleolus, while the nucleus itself is essentially free from mineral residue, except for fine, faintly visible strands, especially along the nuclear membrane (Fig. 9). The mineral in the cytoplasm is globular and granular in arrangement, extending into the apical processes and also into the roots of the dendrites. It corresponds in its arrangement to Nissl bodies. The axone hillock, axis cylinder process and the axone itself are free from mineral, a fact established by Scott.<sup>15</sup> Also, the intracellular neurofibrils are free of mineral. Most of the ash is white, some of it bluish; most of the white ash is insoluble in water. Here and there are granules, but never entire Nissl bodies, in which a slight yellowish tinge indicates the presence of iron. These iron-containing granules are mixed in with the white and bluish granules, frequently in the same Nissl body. There are no Nissl bodies entirely made up of yellowish (iron-containing) granules. The iron-containing granules are less numerous in cortical ganglion cells than in motor ganglion cells of the spinal cord, but even there they are not frequent and are far outnumbered by iron-free granules.

The pyramidal cells of the fascia dentata of the hippocampus are outstandingly rich in all minerals (Fig. 4).

In normal cerebellar cortex the richest mineral deposits are found in the granular cells, while the Purkinje cells contain a comparatively sparse amount in their nucleolus, cytoplasm and dendrites (Figs. 5, 6, 7 and 8). The molecular layer contains but little mineral deposit (Fig. 5) located in the glial Bergmann processes, in sparse glial nuclei and in the nucleoli and faintly visible, short processes of scattered small ganglion cells of the molecular layer (Fig. 6). In suitable preparations long dendrites of the Purkinje cells are well outlined by their mineral content (Fig. 7); the axis cylinder, however, is always invisible, being free of mineral. The intracellular neurofibrils are free of mineral. In some instances Cajal's baskets



are well outlined by the mineral granules they contain (Fig. 8). A part of them is made up of myelinated fibers (Obersteiner<sup>80</sup>). Others are probably dendrites from basket cells, since axis cylinders themselves do not contain mineral ash.

The finding of richer mineral deposits in granular cells in the cerebellum is in contrast to that in the cerebrum where they contain the least amount of mineral. This indicates that granular cells in the cerebrum and cerebellum are fundamentally different in their chemical composition.

In 2 cases of diabetic coma (diabetes mellitus), which we examined, the cerebellum gave in all its parts a typically normal incineration picture. Therefore, we are unable to confirm the statements of Scheid<sup>66</sup> and Wulf<sup>51</sup> concerning a diminution or disappearance of mineral matter from the granular layer of the cerebellar cortex in this condition.

In the spinal cord large motor ganglion cells of the anterior horn stand out by the substantial mineral deposits in their cytoplasm and nucleolus (Figs. 2, 10, 11, 12, 13), while the nucleus is free of them, except for faintly visible fine strands found especially along the nuclear membrane. The mineral deposits in the cytoplasm are globular and, as demonstrated by stained control sections, correspond in their arrangement to Nissl bodies (Figs. 10, 11, 12). The dendrites are also rich in minerals (Fig. 10), while the axis cylinder and axone hillock have none (Fig. 13). Intracellular neurofibrils in normal cells are also free of minerals. That within the Nissl bodies is granular in nature. Most of these granules appear white in darkfield, some bluish. Most of the white granules are insoluble in water. In addition to these the Nissl bodies contain a few granules which by their yellowish tinge indicate the presence of iron. These yellowish granules are somewhat more numerous in motor ganglion cells of the spinal cord than in cerebral cortex. The nucleus is free of iron. The nucleolus is composed of dense, white, water insoluble ash, some of it doubly refractive, as already observed by Scott<sup>15</sup>; whether or not the nucleolus also contains iron cannot be decided with certainty.

Nicholson<sup>81</sup> in 1923, working with Macallum's staining methods, demonstrated the iron content of Nissl bodies. He demonstrated the presence of organically bound iron in the nucleolus, the oxychromatin of Heidenhain and in the chromophilic substance of the

cytoplasm (Nissl bodies). He considers the stainable substance of Nissl, for the most part, an iron-containing protein. Scott<sup>15</sup> confirmed the presence of iron in Nissl bodies by microincineration, pointing out, however, the comparatively larger amount of other minerals they contain. Policard<sup>82</sup> had pointed to the fact that nuclei in adults, on the whole, do not contain enough iron to show yellow coloring by microincineration. We can confirm this definitely for the nucleus of the adult ganglion cell.

The glial ground net and the nuclei and processes of glia cells in the gray matter of the spinal cord contain a fair amount of mineral (Figs. 12, 13, 14), more than the white matter (Fig. 2).

The following examples of pathological changes in anterior horn cells are taken from a series of animal experiments done in collaboration with Dr. W. E. Patton. In cats, which have been subjected to experimental dehydration for a series of days, anterior horn cells show severe changes. The globular mineral deposits undergo dusty degeneration (Fig. 15) and, in some instances, deteriorating ganglion cells are clasped by proliferating neuronophagic oligodendroglia and microglia, the nuclei of which are extremely rich in mineral (Fig. 16). In some instances, mineral deposits of affected ganglion cells shift from the globular Nissl bodies into the interglobular spaces that are normally occupied by neurofibrils (Fig. 14). In later stages the centers of the cells become demineralized, while minerals accumulate along the edges of the cells (Fig. 17). The affected cells become rounded out and present a swollen appearance, comparable to that of pellagral cell disease. In later stages the cells become swollen, rounded and completely demineralized (Fig. 18), with the exception of a few granular deposits along the edges of the cells.

The nerve cell changes in senile dementia, especially Alzheimer's and Pick's disease, will be described later.

#### NORMAL AND PATHOLOGICAL EPENDYMA IN MICROINCINERATION

Normal ventricular ependyma (Fig. 21) is rich in minerals contained mostly in the nuclei of ependymal cells. Most of the ash is white and insoluble in water. Partial depletion of cells, as described by Policard, Morin and Nétik<sup>83</sup> for secretory cells in the pancreas, was never observed by us in the ependyma.

In granular ependymitis (in dementia paralytica, and also in Wernicke's superior hemorrhagic encephalitis in chronic alcoholism with beginning delirium tremens), the mineral content of the ependyma increases greatly, especially in the ependymal villi and in the plaques with increased numbers of rows of ependymal cells (Fig. 23).

The finding of abundant mineral deposits in the ependyma is interesting. Schoenholz<sup>45</sup> found in young placentas that the epithelium was richer in minerals than the stroma. Policard and Bonamour<sup>84</sup> found the anterior and posterior epithelium of the cornea much richer in minerals than the cornea itself. Policard<sup>85</sup> found vacuoles in the endoplasm of Vorticellae lined by mineral deposits. Horning and Scott,<sup>86</sup> comparing the two types of holophytic (saprozoic) and holozoic protozoal infusoria, found that in *Opalina* (a holophytic infusorian) only the dispersed vegetative granules of the endoplasm contained mineral ash, while in *Nyctotherus* (a holozoic infusorian) the vacuoles of the endoplasm and the oral cavity were lined with mineral deposits.

### AMYLOID BODIES

Amyloid bodies are rich in minerals and stand out as homogeneous, bright, highly mineral-containing bodies along the edges of the central nervous system (Fig. 20). Most of the ash of amyloid bodies is white, some of it bluish. Here and there are amyloid bodies in which a slight yellowish tinge indicates the presence of traces of iron. Most of the white and yellowish ash is non-soluble in water. Although their iron content is only slight and restricted, it was successfully demonstrated by von Lehoczky,<sup>87</sup> and confirmed by Merritt, Moore and Solomon,<sup>88</sup> in the brains of paretics and non-paretics.

### MINERAL STRUCTURE OF CEREBRAL BLOOD VESSELS

The mineral structure of cerebral blood vessels is one of the most problematic and controversial subjects in the literature concerning microincineration. No completely conclusive description of mineral in the various layers of blood vessels, either normal or pathological, has yet been given. Probably one of the reasons is the difficulty in technique, especially that of irregular shrinkage. Policard and

Ravault<sup>89</sup> recommend boiling blocks for 1 to 2 minutes in 95 per cent alcohol after fixation, but by careful dehydration and embedding we have been getting satisfactory results without this procedure. The other difficulty is one incurred in obtaining complete incineration of extremely tough and heat resistant fibrous tissue. In working on blood vessels one will have to discard many specimens; only carefully cut, even thin sections give reliable pictures. No incompletely incinerated preparation should be used; remaining carbon may severely distort, sometimes almost invert the darkfield picture.

The first description of incinerated blood vessels was given by Ravault.<sup>90</sup> He found the ashes of the vessel wall to be made up of calcium, some magnesium, and possibly very little silica. According to his findings most of the ash is located in the media, while the intima and adventitia contain less. These minerals were found to really impregnate the structures of the media; they are not amorphous mineral deposits as, for instance, those in an atheroma. With great reserve the author identifies the mineral deposits with the elastic lamellae (page 46) and says: "These ashes are arranged in fine stripes oriented parallel to the elastic layers of the aortic wall. One has the impression that the calcium shown by incineration is actually incorporated in the tissue and one cannot keep from superimposing this mineral skeleton to the elastic framework of the mesaorta." However, Ravault warns against making too definite a conclusion, since possibly *intra vitam* this calcareous impregnation may be diffuse and equally distributed over the elastic fibers and interlamellar substance. In a later paper on pathological changes of the vessel wall Ravault<sup>91</sup> found that in calcification of the media precipitation of calcareous substances occurs as the conclusion of profound alterations of the vessel wall, affecting the muscle fibers, elastic layers and ground substance as well. Schultz-Brauns<sup>41</sup> also speaks of an increase of ash in the elastic fibers. Zinkant<sup>46</sup> found increased ash deposits, mostly calcium, in the elastica interna of uterine arteries from multiparous females in the fifth decade of life in incinerated sections 15  $\mu$  in thickness, and says that "the accumulation of inorganic substances occurred especially and most markedly in the media, in many instances also especially in the elastic layers of the internal elastic membrane." In contrast to these findings Ham<sup>92</sup> found that the normal aorta of rats contained but little mineral matter. His Figures 1 B and 2 B, from the aorta of a

normal rat and from that of a rat 24 hours after administration of activated ergosterol, show the elastic fibers to be free of mineral. Later, however, in hypervitaminosis D, he saw the elastic fibers also become involved in the process, and on occasion show heavy infiltration with minerals. In other places, however, Ham speaks rather of incrustation on, or along, the elastic fibers. One year later Ku<sup>93</sup> considered the ash of normal coronary arteries to be derived principally from elastic fibers, but also in small part from the intima, media and adventitia. This ash is composed almost entirely of calcium salts. In a 46 year old female (Case 8, Table V), however, he describes two kinds of ash: one faintly illuminated, located in the fibrous parts of the intima; the other brightly illuminated, located in the elastica.

In spectroscopic studies Policard, Morel and Ravault<sup>94</sup> found calcium and magnesium in normal and pathological blood vessels, thus confirming earlier chemical work of Baldauf.<sup>95</sup> From their detailed studies they concluded that normal intima contains more calcium than magnesium. The media contains more calcium and much more magnesium than the intima. In lipoid degeneration of the intima calcium increases, but not magnesium. In the media underlying atheromatous plaques both calcium and magnesium are diminished. In calcified atheromata calcium is increased but not magnesium. Pathological mineral deposits in the aorta are always calcareous, the magnesium does not participate in this process but, on the contrary, tends to diminish in these lesions. The authors do not give data on the elastica and in a review in Cowdry's handbook on arteriosclerosis Policard<sup>96</sup> gives none.

In our studies we found most of the mineral of the vascular wall in collagenous fibrous tissue, less in muscle, and still less in reticulin, while elastic tissue was always found to be free of mineral residue. Most of the mineral deposits are insoluble in water, white, and only slight bluish deposits are present. The deposits are richest in the media, but are somewhat less in amount in the intima and adventitia. The complete absence of mineral in elastic tissue is of interest. So far only two other tissues have been found to be free of mineral, namely the axis cylinders of the nervous system (Scott,<sup>15</sup> confirmed by our studies) and the isotropic J bands of striated muscle (Scott<sup>97</sup>).

The wall of a normal meningeal artery contains a considerable

amount of mineral in all layers except the elastica interna, which stands out as a dark, winding narrow band, free of mineral ash (Fig. 22).

In various diseases of the vessel wall, such as arteriosclerosis with proliferation or hyalinization of the intima, hyalinization of the media, or syphilitic endarteritis, the internal elastic membrane always retains its character as an ash-free structure.

Figure 24 shows an oblique longitudinal section through a larger branch of the arteria chorioidea, taken from its extracerebral course near the Ammon's horn from a case of cerebral arteriosclerosis (a 56 year old male). The mineral residue is most dense in the media, while the newly formed intimal proliferation contains somewhat less. The inner elastic membrane, although in some places delaminated, can still be seen as a dark, non-mineral-containing layer, which in places of delamination is split into two or three equally dark, ash-free layers. In between the layers mineral-containing, newly formed collagenous fibrous tissue can be seen. In many places the mineral-free, inner elastic membrane, especially its inner lamina, is seen to be perforated by many fine, mineral-containing collagenous fibers which connect the inner intimal proliferation with the outer part of the intima and the media. This confirms Ranke's<sup>98</sup> findings of fenestrations of the elastic membranes, and especially his findings concerning the increase of these fenestrations in arteriosclerosis and related vascular diseases. In thrombosed vessels the fine network of fibrin, and later the network of reticulin, contains very little mineral residue (Fig. 27, upper half of picture). The nuclei of the emigrating fibroblasts and endothelial cells, however, the newformed collagenous fibrous tissue, and especially the lining of recanalized tissue, is richer in minerals (Fig. 27, left lower quarter of figure). Most rich in mineral, however, are the large siderophagic scavenger cells, which take up the hematogenous iron from the clot and stand out by reason of considerable amounts of ingested dark yellow and red iron oxide (Fig. 27, right lower quarter of figure, near the wall of the vessel). These cells frequently are found also to contain lipoid, as found by us in collaboration with Dr. T. J. Putnam, and thereby resemble Ciaccio cells (Baginski<sup>99</sup>).

## MINERAL STRUCTURE OF VASCULAR LESIONS OF THE BRAIN

*Mineral Residue of Hemorrhages, with Reference to the Mineral Content of Various Elements of the Blood*

Staining methods do not reveal the iron of hemoglobin (Macalium<sup>100</sup>). Policard<sup>33, 35, 101</sup> and Policard and Pillet<sup>102</sup> found that although the single red cell does not leave enough ash of sufficient density to be sure of its iron content, agglomerated masses of cells from a fresh smear or in fluid blood in blood vessels leave after incineration a definitely yellowish, iron-containing ash.

In our experience with blood smears two elements stand out by their massive mineral content — the white blood cells and the blood platelets (Fig. 32). The white cells, leukocytes as well as lymphocytes, show dense white mineral deposits in their nuclei; the leukocytes relatively more than the lymphocytes. The cytoplasm of the polymorphonuclear leukocytes shows only a few, fine, faintly stainable bluish granules. According to Scott<sup>15</sup> the eosinophiles may be recognized by white cytoplasmic ash granules. Blood platelets are conspicuous by their particularly dense, brilliant, water insoluble ash. The red cells contain only fine, faintly visible mineral granules, most of them accumulated along the edges of the red cells which thereby, in many instances, appear as rings in the darkfield (Fig. 32). Most of these granules are of a bluish color; only here and there are fine yellowish granules visible. Figure 19 is taken from a case of myeloid leukemia and illustrates the high mineral content in the nuclei of myeloblasts, myelocytes and leukocytes. A smaller amount of mineral is seen in the cytoplasm of these cells. In Figure 19, surrounding the large myeloblast, many blood platelets are seen which are clearly composed of small, regularly distributed mineral granules corresponding to azurophilic granules, a fact that had been observed by Scott.<sup>15</sup>

Perivascular hemorrhages in the brain are conspicuous by their content of yellowish iron-containing mineral (Fig. 25); the yellow color increases in depth as the chemical transformation of hemoglobin into hematoïdin and hemosiderin proceeds; by the time of ingestion by scavenger cells the color has changed to orange or deep red.

*Mineral Structure of Softening and of Ischemic Necrosis*

Foci of softening and ischemic necrosis of the brain stand out as grossly demineralized areas in microincinerated preparations. In the focus of softening (Fig. 26) only scavenger cells and remaining vascular network in the form of engorged, medium sized blood vessels can be seen. The scavenger cells especially stand out as isolated bodies of high mineral content in an otherwise completely demineralized field. Foci of ischemic necrosis show all gradations from complete demineralization of nervous tissue up to a marked or slight diminution of minerals in a more or less sharply circumscribed area. The time required for this demineralization in ischemic necrosis is surprisingly short; we have seen it in lesions of only 1 days duration. The manner in which mineral is removed cannot be determined.

This demineralization in vascular lesions of nervous tissue has certain parallels in bone and in the placenta. Policard,<sup>103</sup> and Policard and Péhu<sup>104</sup> found that during the process of ossification demineralization of the ground substances of cartilage takes place in regions devoid of blood vessels. The authors wonder if this possibly may be due to edema. Schultz-Brauns and Schoenholz,<sup>44</sup> who found such demineralization in the placenta, thought it due to the fact that easily soluble salts, after the death of the cells, are immediately dissolved and removed, the body as a whole being undersaturated in soluble salts. A fully satisfactory explanation, however, has not yet been given.

## MINERAL IN TUMORS OF THE BRAIN

Policard and Doubrow<sup>9</sup> were impressed by the fact that tumor tissue, by the microincineration process, remained carbonized much longer than normal, but considered it, however, on the whole to be rather poor in mineral ash. Scott and Horning<sup>105, 106</sup> discovered the important fact that tumor tissue, when compared with similar normal tissue, contains a relatively much greater amount of mineral ash, especially of calcium and iron oxide. The nuclei especially contain more ash than the nuclei of normal tissue. The nuclear ash contains also more iron oxide than normal cells, but the cytoplasmic ash is more abundant. In all these features of distribution and arrangement of mineral salts malignant tumor tissue resembles that of the



developing chick embryo (Horning and Scott <sup>79</sup>). The authors see in this a contribution to Cohnheim's embryonal theory of tumor proliferation. In this connection it is of great interest to remember that the water content of tumor tissue, as well as that of embryonic, is higher than that of normal tissue (Schlottman and Rubenow <sup>107</sup>). The water content of pig embryos increases up to 160 mm. length, then gradually decreases until birth (Wilkerson and Gortner <sup>108</sup>).

We are able fully to confirm Horning's and Scott's findings on our material of tumors of the brain, gliomatous as well as metastatic. Even at low power tumors in incinerated preparations can be seen at once to be extremely rich in mineral-containing substances, in contrast to normal brain tissue (Fig. 30). The mineral accumulates especially in nuclei, less in the cytoplasm of tumor cells (Fig. 31). Most of the mineral granules appear white or yellow, fewer are bluish in darkfield; most of the white calcium-containing and yellow iron-containing minerals are insoluble in water. The mineral content of the tumors may possibly play a rôle in their X-ray sensitivity.

## MINERALS IN INFLAMMATORY LESIONS OF THE BRAIN

### *Purulent Meningitis and Meningoencephalitis*

In purulent meningitis the meningeal exudate is extremely rich in minerals, in contrast with which the brain appears rather dark and poor in minerals (Fig. 28). The mineral of the exudate is mainly accumulated in the nuclei, less in the cytoplasm of polymorphonuclear leukocytes and meningeal histiocytes, and in the nuclei of lymphocytes. Most of the mineral residue is whitish and insoluble in water, less of it bluish and soluble in water. Similar mineral deposits are found in the polymorphonuclear and histiocytic perivascular infiltrations around intracerebral cortical blood vessels in lesions of purulent meningoencephalitis (Fig. 29).

### *Syphilitic Meningoencephalitis, Gumma and Dementia Paralytica*

The inflammatory lesions of syphilitic meningoencephalitis, gumma and dementia paralytica stand out by their extremely rich mineral deposits; the degenerative lesions of dementia paralytica, however, as well as the areas of infarction in syphilitic meningoencephalitis, are characterized by demineralization of nervous tissue.

Iron occurs in microincinerated preparations of neurosyphilitic lesions in two forms: (1) concentrated as dark yellow, orange or red deposits of granules of iron oxide in (a) histiocytic cells of meningeal infiltrations, especially around blood vessels; (b) histiocytic and microglial elements of intracerebral perivascular cuffs, especially in abundance near gummas; (c) isolated perivascular histiocytes and microglial cells throughout the brain, especially the gray matter; and (d) microglia cells in focal lesions. These iron deposits do not consist of hemosiderin and are invisible in non-incinerated or unstained preparations. (2) It is also found as an admixture to other minerals as light yellow, granular mineral deposits, indicative of the presence of iron oxide in (a) gummas, in the remains of the nuclei of many of the infiltrative cells as well as in the ground substance of the gumma; (b) nuclei and cytoplasm of certain plasma cells in perivascular infiltrative cuffs; (c) nuclei of certain lymphocytes of perivascular infiltrative cuffs; and (d) nuclei and processes of microglia cells in and around lesions.

Most of the infiltrating lymphocytes and plasma cells are free of iron, as are all macroglial astrocytes, including those that show active growth and proliferation in and around lesions. These cells contain only whitish and bluish ash. This ash is contained in the nuclei of lymphocytes, plasma and macroglial cells. It is also seen in the cytoplasm of these cells but not in abundance except in the cytoplasm of the proliferating astrocytes, which contain a great deal of ash.

Perivascular syphilitic infiltrations, especially in the region of gummas, show the difference in iron content between the various infiltrative elements most impressively. Some of these infiltrations show heavy, dark colored iron deposits only at one particular point at the periphery of the perivascular infiltration. Stained control sections reveal that these deposits are exclusively contained in histiocytic and microglial elements which accumulate at this point. The centers of such dense perivascular infiltrations reveal only faintly yellowish granules, indicating traces of iron oxide in the nuclei and cytoplasm of a few plasma cells and in the nuclei of a few lymphocytes. The majority of the lymphocytic and plasma cell infiltrations contains whitish and bluish mineral granules in the nuclei and in the cytoplasm of the latter.

Gummas are among the most highly hypermineralized lesions of

the nervous system. At low power (Fig. 33) they stand out as light glistening areas, owing to their extremely rich mineral content. The mineral within the gumma is also much denser in its distribution than the mineral in normal tissue or even in tumor tissue; its structure, however, is not quite homogeneous, but definitely granular. At higher power the gumma appears to be composed of larger and smaller granules, arranged somewhat regularly. The larger granules are composed of dense aggregates of small, yellowish and white granules corresponding to remains of nuclei and nuclear fragments of the original gummatous infiltration. The small granules are rather evenly distributed in between and are composed of light yellowish, whitish and bluish granules: in some places yellowish ashes, in others bluish predominate. In addition to these granules the incinerated preparations reveal also fine mineralized fibers, most of them toward the edges of the gumma. Some of them are primitive, non-collagenous fibrous tissue fibers, others are processes of microglia cells, which can be seen in active proliferation at many places along the edges of the gumma. Ramified microglial elements can be identified. Also, a few macroglial astrocytes are seen here, recognizable by their plumper shape and exclusively white and bluish mineral content. We have not yet been able to identify spirochetes in microincinerated preparations.

### MINERALS IN MULTIPLE SCLEROSIS

The plaques of multiple sclerosis are demineralized lesions. In microincinerated preparations at low power, they stand out as dark areas, owing to the fact that their ground substance has become void of mineral salts (Figs. 34, 35, 36, 37). The engorged blood vessels of early hyperemic plaques, however, stand out as brightly illuminated spots (Figs. 37, 40), and the meshes of the glial network and macroglial astrocytes are well outlined as light, mineral-containing bodies against the black background of the plaque (Figs. 39, 40).

The engorged small blood vessels and capillaries, many of which contain early thrombi, show in their adventitial spaces, in some instances, in addition to lymphocytic-histiocytic infiltration, large phagocytic cells that are rich in reddish yellow granules of iron oxide (Figs. 38, 40). Many of these same cells, as shown by control slides,

also contain lipoid material. The admixture of minerals with lipoids, which also occurs in Ciaccio cells (Baginski <sup>99</sup>), may furnish part of the crystalline appearance of cholesterol esters, described by Amorim <sup>109</sup> in phagocytic cells around blood vessels in lesions of the nervous system.

Especially impressive is the appearance of macroglial protoplasmic astrocytes in microincinerated preparations of these lesions (Figs. 39, 40). Their nuclei, cell bodies and processes with all their ramifications, are well outlined by a rich mineral content. The nuclei, however, contain only slightly more or less mineral than the cell bodies and, therefore, in many instances differentiation is not clear (Fig. 40). The minerals consist of dense, whitish and bluish granules, the former being in the majority. Most of the white granules are insoluble in water and do not contain iron oxide. This picture presented by macroglial astrocytes in incinerated preparations in its completeness compares well with specimens prepared by Cajal's gold sublimate method.

In the older anemic plaques, which stand out as grossly demineralized areas (Fig. 41), many smaller blood vessels have undergone obliteration, while on the demineralized background of the plaque, well outlined, large, mineral-containing fibrillary astrocytes can be seen (Figs. 41, 42). These findings prove that demineralization is an additional, essential feature in the pathology of these lesions which have customarily been referred to as demyelinating. This term, however, takes into account only one part of the total picture, which is characterized by demineralization as well.

It is of great interest to find that the plaques in multiple sclerosis in their mineral architecture most closely resemble foci of softening or of ischemic necrosis, all these being essentially lesions of demineralization. These findings tend to confirm the observations of Putnam,<sup>110, 111</sup> who described the multiple sclerotic plaques as regressive lesions of vascular origin.

## MINERALS IN SENILE DEMENTIA

### *Pick's and Alzheimer's Disease*

In the cortical disease of senile dementia loss and damage of ganglion cells, disarrangement of cortical architecture and gliosis are well recognizable in incinerated preparations. Areas depleted of

ganglion cells appear also demineralized by their loss. The alteration in shape of ganglion cells is well recognizable, also the condensation of their mineral deposits in shrinkage (Fig. 48). These mineral deposits are white, most of them insoluble in water. The nuclei of glia cells, and to less extent their cytoplasm and processes, are well outlined by their mineral content; places of intense gliosis show at low power a local increase of mineral. The small glia rosettes which clasp or replace necrotic ganglion cells (Fig. 48) are well recognizable by the dense mineral content present in the nuclei and in a few of the processes of the small groups of oligodendroglial and microglial cells that make up these rosettes. Most of their mineral content is white and insoluble in water, less of it bluish.

Figure 47 shows an incinerated section from the area entorhinalis of a case of Pick's disease in a 72 year old female. The pathology of this area in diseases of the cerebral cortex, including Pick's disease, has recently been described by Rose.<sup>112</sup> We are able to confirm Rose's findings in our microincinerated preparations. The disturbance of cortical architecture is obvious at low power (Fig. 47). Most conspicuous is the almost complete loss of ganglion cells in the outer part of the lamina principalis externa (*Pre-Alpha*); somewhat less complete is the loss of ganglion cells in the lamina principalis interna (*Pri*), while in the inner part of the lamina principalis externa (*Pre-Beta* and *Gamma*) and in the lamina dissecans (*Ds*) a great many ganglion cells are preserved. Many of them, however, show pathological changes such as shrinkage with condensation of mineral matter, corkscrew deformity of processes and in some instances complete pyknosis. These layers show also considerable gliosis, especially of microglial, but also of oligodendroglial cells. Many of the processes of microglial cells are faintly visible, even at this low power, owing to their content of whitish, bluish and yellowish mineral deposits.

The senile plaques in senile dementia, and especially in Alzheimer's disease, when subjected to microincineration burn out completely and do not leave any appreciable traces; their site is neither marked by excess mineral residue, nor by demineralization. This is well illustrated by Figures 43 and 44, which are neighboring sections from a series of the cornu ammonis (field H 4 and fascia dentata) from a case of Alzheimer's disease in a 74 year old female. Figure 43 is stained with a modification of Bielschowsky's silver impregnation

for paraffin sections; Figure 44 is microincinerated. The cellular ribbon of the fascia dentata in the lower half, and the blood vessel in the left lower corner of each picture are landmarks that make orientation easy. In the Bielschowsky stained Figure 43 three senile plaques are well shown: one is adjacent to the dorsal surface of the fascia dentata, and two are in the upper half of the picture in field H 4. In the microincinerated neighboring preparation (Figure 44) no trace of these plaques is visible at low power; they do not stand out either as areas of increase or of decrease of tissue minerals. Figures 45 and 46 show one of these plaques, the one adjacent to the fascia dentata, at higher power. In Figure 45, a Bielschowsky stained preparation, the plaque appears clearly, dorsad from the cellular ribbon of the fascia dentata, and to the left of a small blood vessel. In Figure 46, a microincinerated preparation, the cellular ribbon of the fascia dentata, the small blood vessel and the other landmarks of the tissue are equally well demonstrated but the senile plaque is at first invisible. On closer examination, however, the plaque can be identified to the left of the small blood vessel as a slight disarrangement of the glial reticulum which, however, is neither poorer nor richer in minerals than the rest of the glial ground net of the tissue. These findings tend to disprove von Braunmühl's<sup>113</sup> theory, which considers the senile plaques inorganic mineral precipitates and, to some extent, favor Bouman's<sup>114</sup> theory, which considers them to be metaplasias of the glial reticulum. We conclude from our studies that senile plaques are of proteid or other organic material and do not contain mineral deposits because they burn out on microincinerated slides. Their exact nature has not yet been determined. Hechst<sup>115</sup> and Winkler-Junius,<sup>116</sup> with reference to the work of Bennhold,<sup>117</sup> Divry,<sup>118</sup> and Leupold,<sup>119</sup> stressed the amyloid content of the plaques, which Winkler-Junius considered amyloid degeneration of phagocytic microglia cells, thought to make up the centers of plaques. Recently Gerstmann, Sträussler and Scheinker<sup>120</sup> described atypical formations, closely resembling senile plaques, in a 28 year old patient suffering from a heredodegenerative spinocerebellar ataxia with cortical involvement. However, these formations, although distinguished by increased argentophilia, similar to senile plaques, are stained vividly also with various other stains.

The neurofibrillar strands in Alzheimer's ganglion cell disease,

however, are well demonstrated in microincinerated slides, standing out as amply mineral-containing interlaced strands, while the remainder of the cytoplasm of the cell is demineralized (Fig. 49). The mineral deposits in Alzheimer's neurofibrillar strands are white and for the most part non-soluble in water. Figure 50 shows an interesting group of three ganglion cells from the subiculum of the same case. In the left half of the picture a characteristic Alzheimer cell is seen with, however, only marginal arrangement of the thickened hypermineralized strands. The upper cell in the right half of the picture is about normal; its granuloglobular mineral deposits correspond to the Nissl bodies. The third cell, in the lower part of the right half of the picture, appears normal only in its upper apical part, above the nucleus, while in its basal part the cytoplasm is filled with thickened hypermineralized Alzheimer strands. Bielschowsky<sup>121</sup> was the first to describe pathological cells in Alzheimer's disease which showed Alzheimer strands only in their basal part, near the pigmented spot, while the rest of the cell showed normal neurofibrillar structure. We are able to confirm this finding for the mineral structure also of these cells. Bielschowsky<sup>122</sup> deduced from this finding that Alzheimer's strands are the result of impregnation of neurofibrillar structures with foreign substances. We are able to conclude from our microincineration studies that one of these substances is mineral, since normal neurofibrils are free of mineral, while Alzheimer's neurofibrillar strands contain mineral. The mineral deposits alone, however, cannot be the cause of the argentophilia of Alzheimer's neurofibrillar strands because the senile plaques, although equally argentophilic, are free of mineral. Von Braunmühl<sup>113</sup> considers Alzheimer's strands to be the result of hydration and swelling of previously dehydrated structures. One of us,<sup>123</sup> in collaboration with Wu,<sup>124</sup> has demonstrated that thickened, argentophilic neurofibrillar strands are found in cortical ganglion cells of patients who died from dysentery, cholera or intestinal tuberculosis in a cachectic, emaciated and dehydrated condition and whose brains showed pseudoatrophy and gave evidence of an altered water-binding capacity. It was also shown that somewhat similar, thickened argentophilic neurofibrillar strands could be produced artificially in fresh brain tissue exposed to water or saline solutions of various concentration. To these indications of an altered, possibly relatively increased water content in Alzheimer strands we can

add the new fact of an increased mineral content, clearly demonstrated by microincinerated preparations. The simultaneous increase of both water and mineral content is not incompatible, since in tumor and in embryonic tissue, water as well as minerals is found to be increased; while in ganglion cell disease produced by experimental dehydration, demineralization was observed. These observations, although throwing some light on this condition, do not yet offer a conclusive explanation.

The main conclusions drawn from our data on the microincineration of senile plaques and Alzheimer's neurofibrillar strands are: (1) Alzheimer's neurofibrillar strands in microincinerated preparations differ from normal neurofibrils in that they contain mineral deposits, while normal neurofibrils contain none. (2) Senile plaques and Alzheimer's neurofibrillar strands, which are usually regarded as pathological changes of similar type (Bouman <sup>114</sup>) on the basis of their argentophilia, differ fundamentally in microincinerated preparations as senile plaques are free of mineral, while Alzheimer's neurofibrillar strands contain mineral deposits.

### SUMMARY AND CONCLUSIONS

1. The cerebral cortex of the newborn human infant is rich in minerals (calcium, iron, and certain water soluble minerals). Most of it is contained in the nuclei of the ganglion cells and glial elements of all layers, while the cytoplasm of these cells contains very little or no mineral ash.

2. In the normal adult the ganglion cells of pyramidal type in the cerebral cortex, the Purkinje cells in the cerebellum, and the anterior horn cells in the spinal cord contain considerable mineral deposit in their nucleolus, cytoplasm and dendrites. The nucleus is essentially free from mineral residue except for a few fine, faintly visible strands, especially along the nuclear membrane. The mineral deposits in the cytoplasm are globular and granular in character and correspond in their arrangement to the Nissl bodies. They contain calcium, very little iron oxide, and certain water soluble minerals. The intracellular neurofibrils, axone hillock and axis cylinder are free from mineral ashes.

The granular cells of the cerebellum contain more mineral residue than those of the cerebrum.



The glial ground net, nuclei, and certain processes of glial cells contain a fair mount of mineral, most of it white and for the most part insoluble in water.

3. Dusty deterioration, homogenization and destruction of the Nissl substance in diseased ganglion cells is demonstrated in micro-incinerated preparations to be associated with severe changes of the mineral structure of the affected ganglion cells and ranges from dusty deterioration and shifting of the minerals to complete demineralization.

4. The ventricular ependyma is rich in minerals, most of its ash is white and for the most part insoluble in water. The mineral content of the ependyma increases greatly in granular ependymitis.

5. Amyloid bodies are rich in minerals, especially in calcium; traces of iron are found only at times.

6. The walls of the cerebral blood vessels are rich in white mineral ash which for the most part is insoluble in water. Most of these mineral deposits are found in the collagenous fibrous tissue, less in the muscle tissue, still less in the reticulin, while the elastic tissue was always found to be free from mineral residue. Also, in various diseases of the vessel wall, such as arteriosclerosis with proliferation or hyalinization of the intima, hyalinization of the media, or syphilitic endarteritis, the internal elastic membrane always retains its character as an ash-free structure. Thereby the fenestrations and perforations of the elastic membranes and their increase in arteriosclerosis and related vascular disease stand out conspicuously in microincinerated preparations. In thrombi of various ages fibrin and reticulin contain very little mineral residue; the nuclei of emigrating fibroblasts and endothelial cells, however, as well as the new formed collagenous tissue and the lining of recanalizations, are richer in mineral. Most rich in mineral (most of it iron oxide) are the large siderophagic scavenger cells in and around thrombosed vessels.

7. Cerebral hemorrhages are conspicuous by their content of yellowish iron-containing mineral. The yellow color increases in depth and may change to orange or deep red as the chemical transformation of the hemoglobin into hematoïdin and hemosiderin proceeds.

The mineral content of the various elements of the blood is discussed.

8. Foci of softening and ischemic necrosis are characterized by demineralization. In the former only the scavenger cells and a few

blood vessels stand out as isolated mineral-containing bodies in an otherwise completely demineralized field.

9. Gliomatous and metastatic brain tumors are outstanding as areas of hypermineralization. The mineral accumulates in excess in the nuclei, less in the cytoplasm of the tumor cells.

10. Inflammatory lesions of the nervous system, including those of purulent meningitis, purulent meningoencephalitis, syphilitic meningoencephalitis, gumma and dementia paralytica are characterized by hypermineralization. The mineral is accumulated in the nuclei, in the nuclear fragments, and in the remains of the infiltrative cells, as well as in the cytoplasm and the processes of certain of these cells, especially in those of glial origin. The iron content of certain infiltrative cells in neurosyphilis is demonstrated well in microincinerated preparations.

11. The lesions of multiple sclerosis are characterized by demineralization. Only the engorged and thrombosed blood vessels, the infiltrative cells, including iron-loaded scavenger cells, the macroglial astrocytes and the meshes of the glial reticulum, stand out as the sole remaining mineral-containing bodies in an otherwise completely demineralized area.

12. Areas and layers in senile cortical atrophy, and especially in Pick's disease, which are depleted of ganglion cells appear demineralized by their loss.

13. Senile plaques in senile dementia, and especially in Alzheimer's disease, when subjected to microincineration burn out completely. Their site is neither marked by excess mineral residue nor by demineralization. The only trace of them recognizable in microincinerated preparations is a slight disarrangement of the glial reticulum which, however, is neither poorer nor richer in ash than the rest of the glial ground net of the tissue.

14. The neurofibrillar strands in Alzheimer's ganglion cell disease differ from normal neurofibrils: Alzheimer's strands contain ample mineral deposits, while normal neurofibrils are free from mineral ash. The mineral deposits in Alzheimer's neurofibrillar strands are white and for the most part insoluble in water. The remainder of the cytoplasm of these cells is demineralized.

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## DESCRIPTION OF PLATES

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Unless otherwise stated all sections were cut at  $4\ \mu$ , subjected to the microincineration process, and examined by darkfield illumination.

### PLATE 63

FIG. 1. Newborn premature human infant. Cerebral cortex, area 10, third layer.  $\times 530$ .

FIG. 2. Anterior horn (ah) and anterolateral column (ac) from a normal spinal cord. Cat.  $\times 156$ .

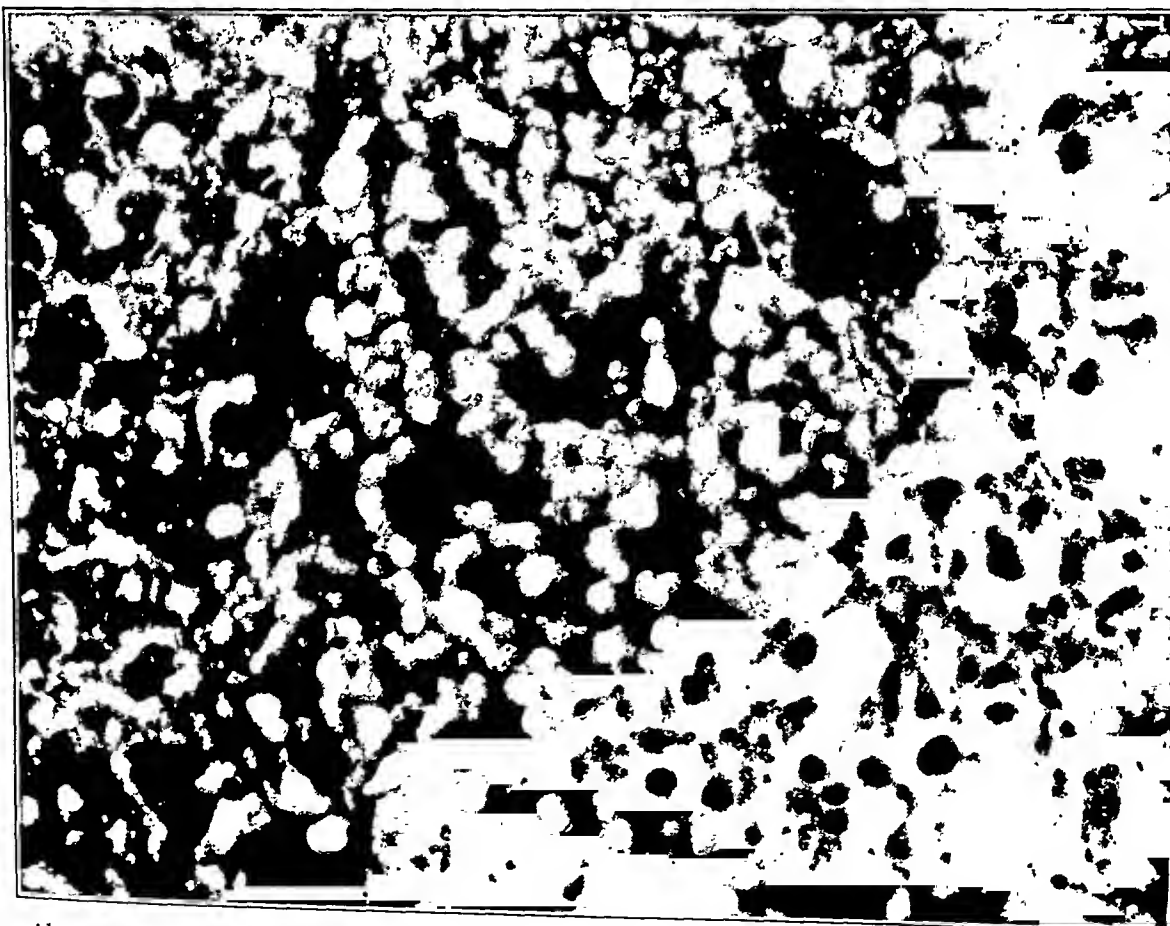
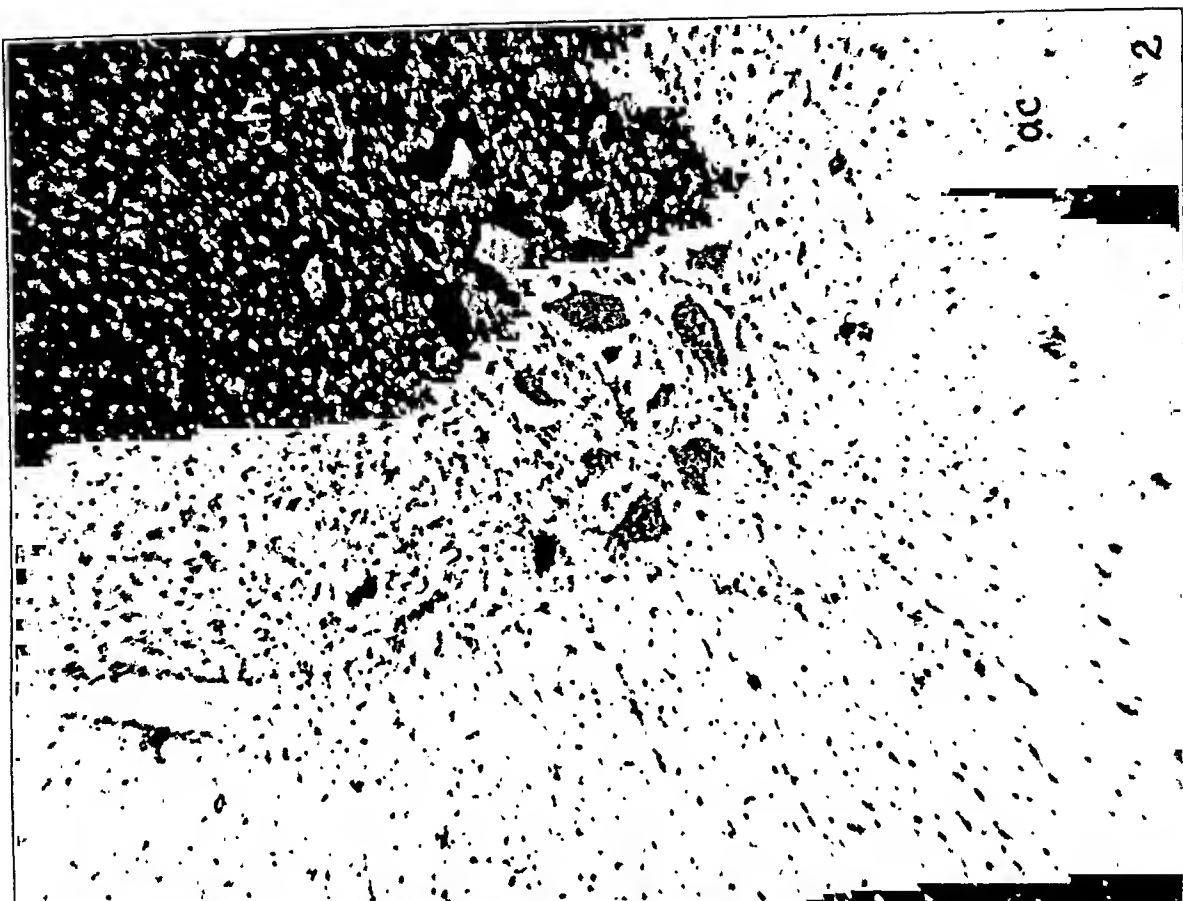


PLATE 64

FIG. 3. Normal adult male. Cerebral cortex, area 10, third and fourth layer.  
× 100.

FIG. 4. The fascia dentata of the cornu ammonis of a normal human adult.  
× 125.

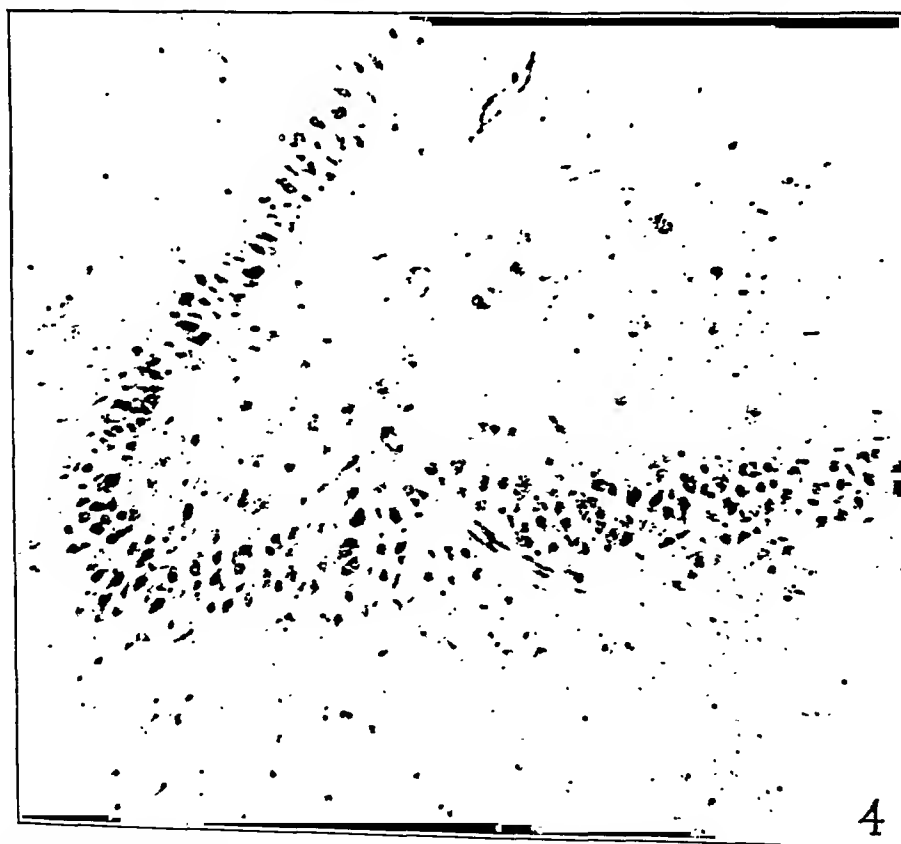
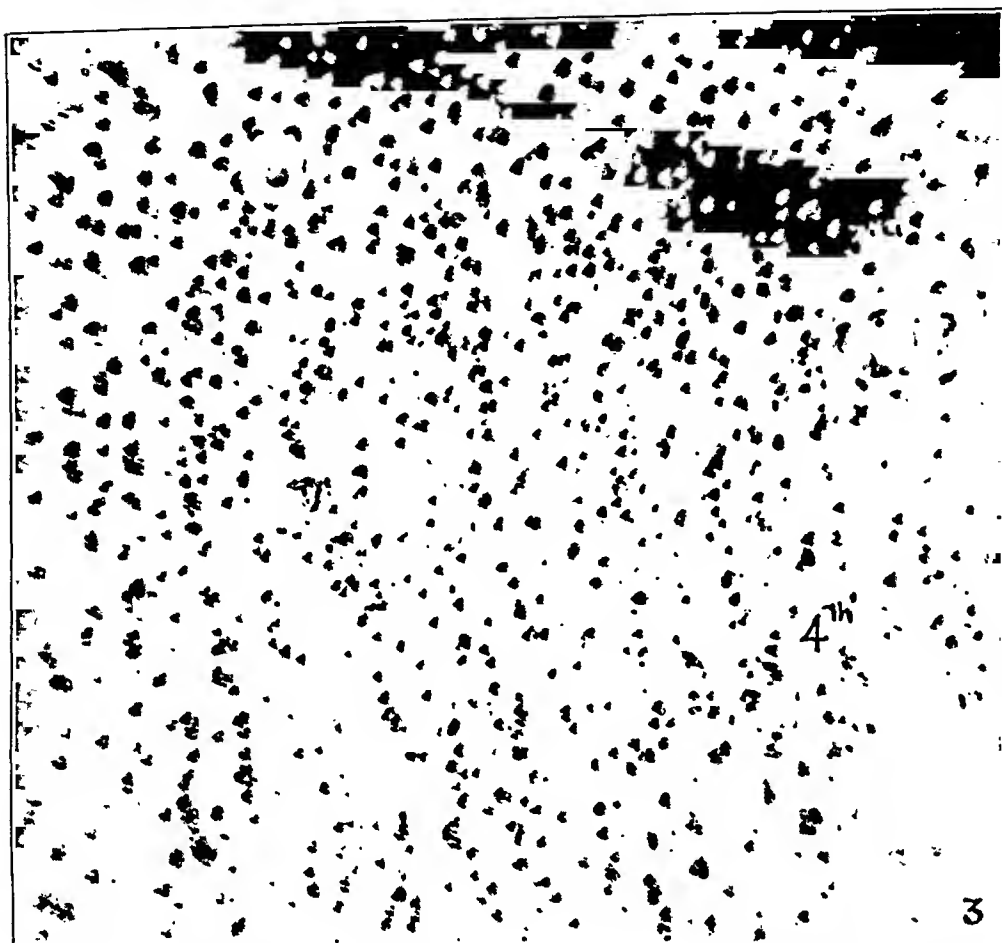


PLATE 65

FIG. 5. The cerebellar cortex of a normal human adult.  $\times 110$ .

FIG. 6. Normal human adult. Cerebellar cortex. m = molecular layer; P = Purkinje layer; g = granular layer.  $\times 576$ .

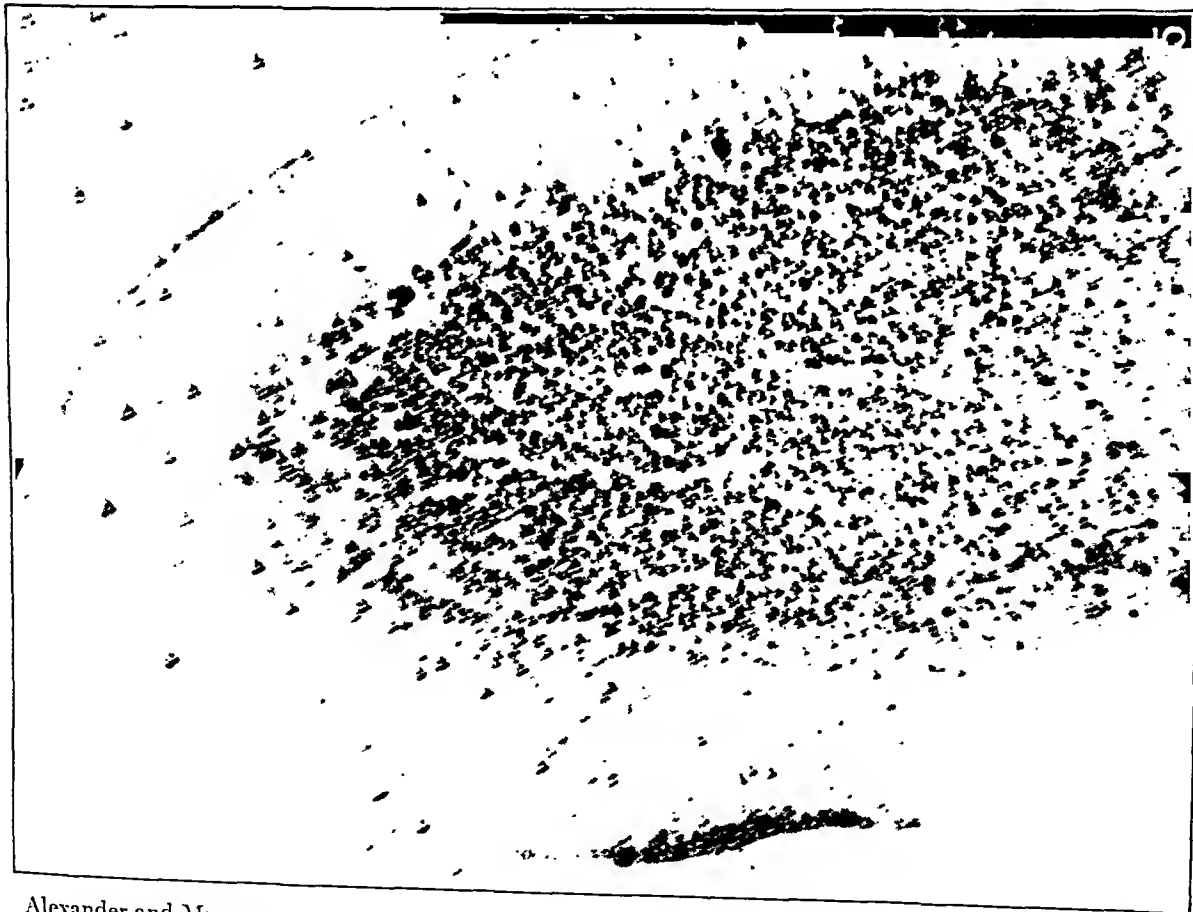
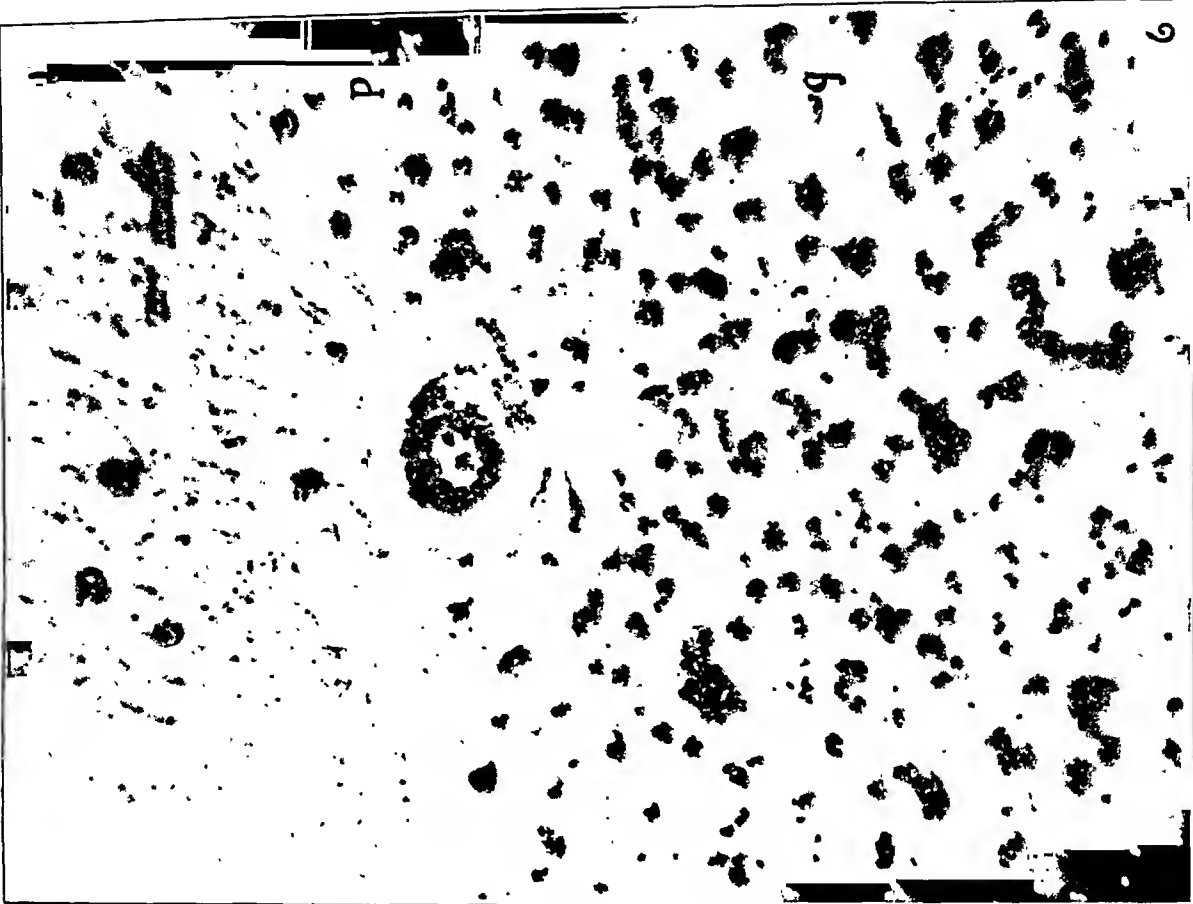




PLATE 66

FIG. 7. Normal human adult. Cerebellar cortex. m = molecular layer; P = Purkinje layer; g = granular layer.  $\times 576$ .

FIG. 8. Normal human adult. Cerebellar cortex. C = Cajal's basket.  $\times 576$ .

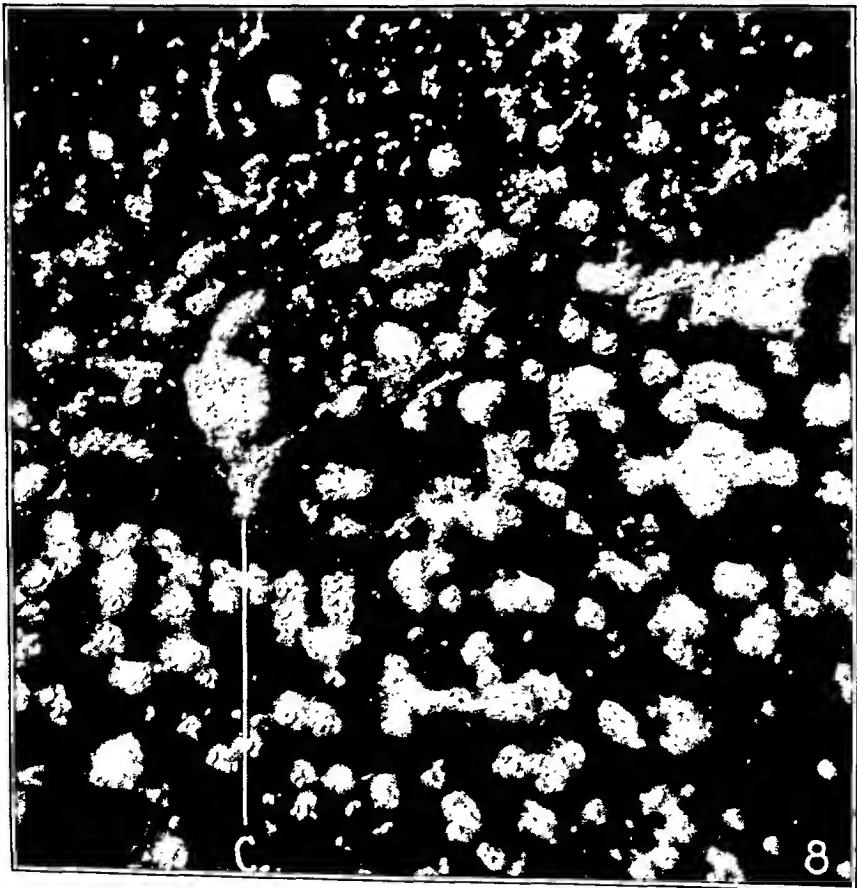
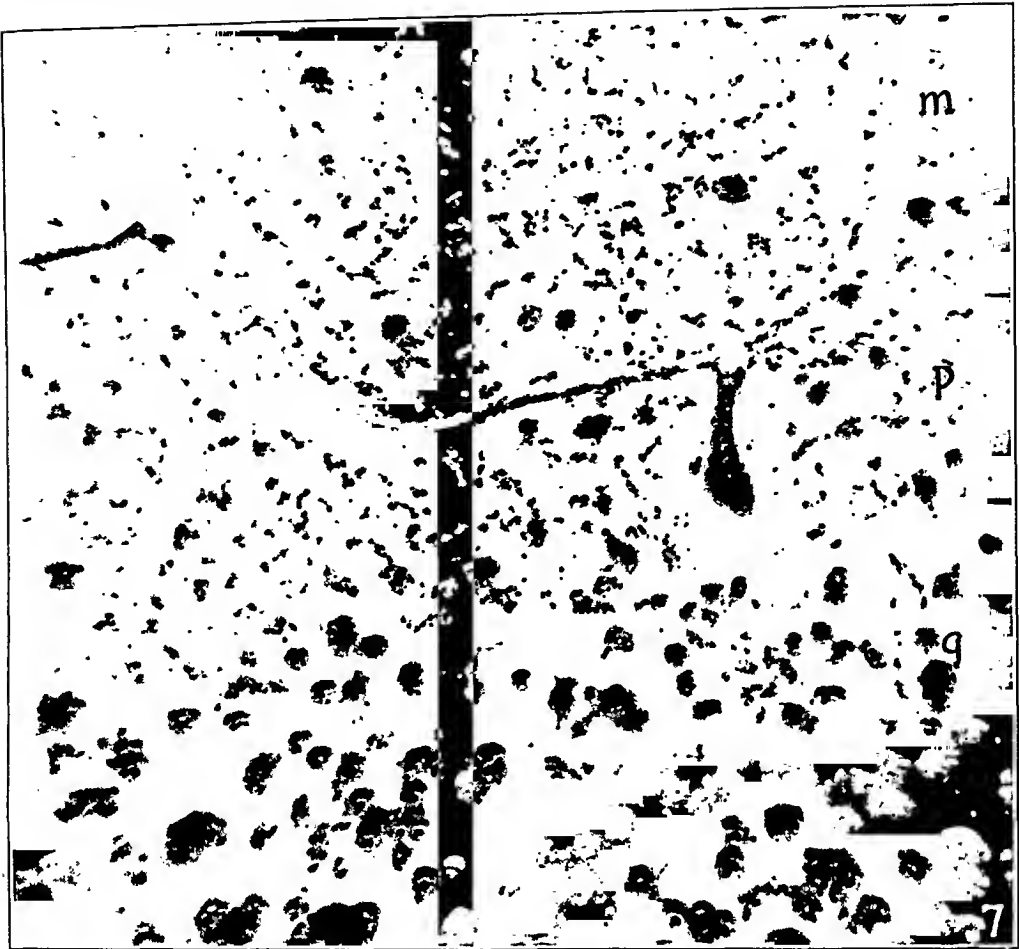
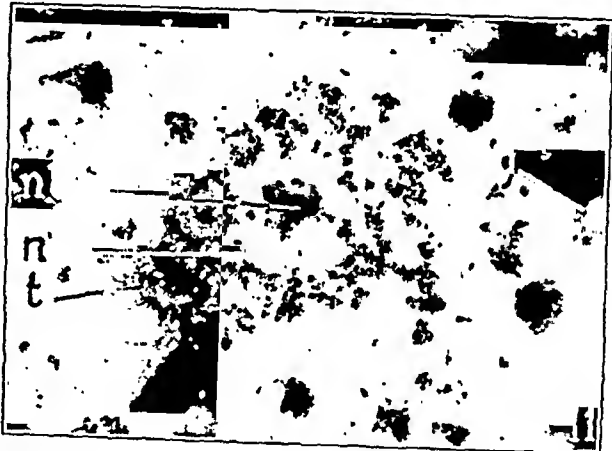
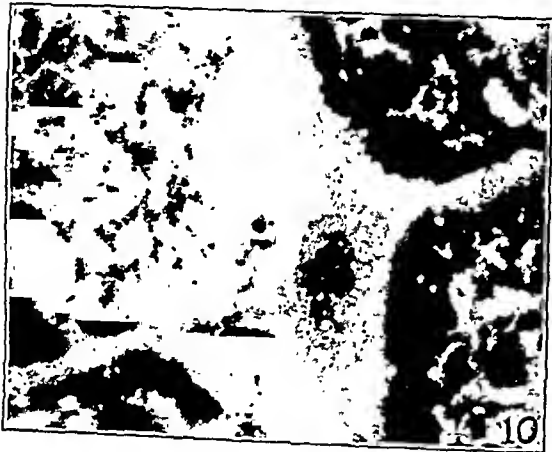
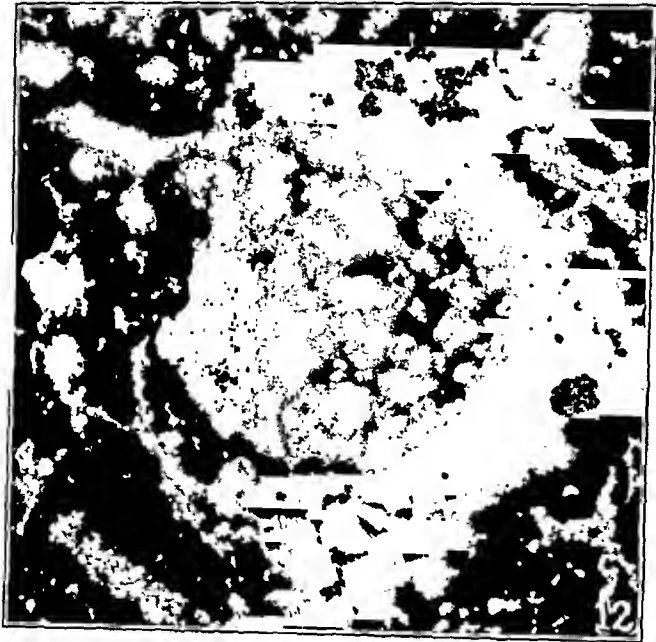


PLATE 67

FIG. 9. Normal pyramidal ganglion cell from the cornu ammonis (field H 2). Human adult.  $\times 760$ .

FIGS. 10, 11, 12 and 13. Normal ganglion cells from the anterior horn of the spinal cord of a cat. n = nucleus; nl = nucleolus; t = tigroid bodies; g = glia cell; ac = axis cylinder.  $\times 760$ .

FIG. 14. Cat, experimental dehydration. Ganglion cell from the anterior horn of the spinal cord. Mineral deposits are present in interglobular spaces.  $\times 760$ .



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Mineral Content of Cerebral Lesions

## PLATE 68

- FIG. 15. Cat, experimental dehydration. Ganglion cell from the anterior horn of the spinal cord. Dusty deterioration of mineral deposits corresponding to tigroid substance of cytoplasm.  $\times 760$ .
- FIG. 16. Cat, experimental dehydration. Ganglion cell (n) from the anterior horn of the spinal cord. Dusty deterioration of mineral deposits, corresponding to tigroid substance of cytoplasm. Cell clasped by oligodendroglia cells (ol).  $\times 760$ .
- FIG. 17. Cat, experimental dehydration. Ganglion cell from the anterior horn of the spinal cord. Central demineralization.  $\times 760$ .
- FIG. 18. Cat, experimental dehydration. Ganglion cell from the anterior horn of the spinal cord. Complete demineralization.  $\times 760$ .
- FIG. 19. A myeloblast and several thrombocytes from a blood smear from a case of myeloid leukemia.  $\times 800$ .

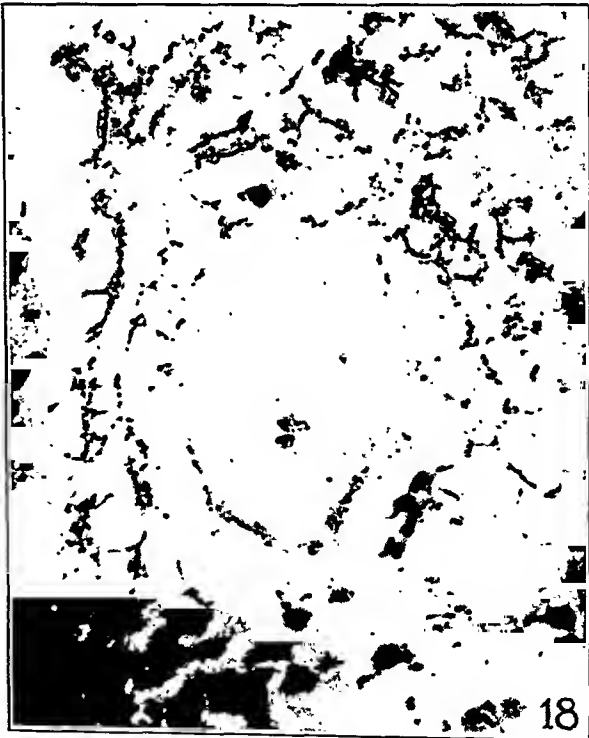
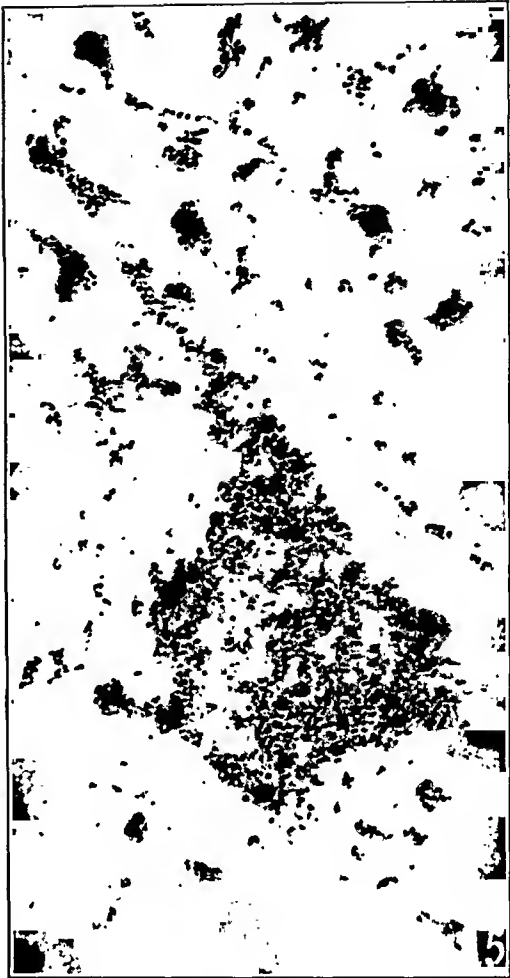
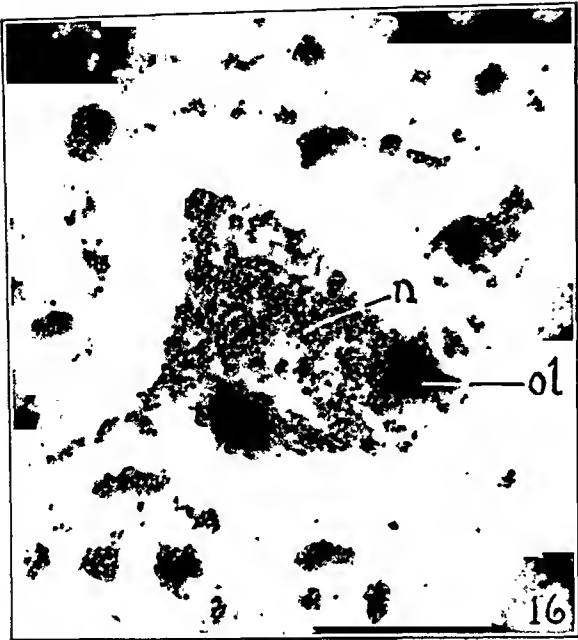


PLATE 69

FIG. 20. Amyloid bodies in the marginal glial layer of the gyrus hippocampi of a 56 year old male with cerebral and generalized arteriosclerosis. am = marginal glial layer, containing amyloid bodies; s = subarachnoid space.  $\times 115$ .

FIG. 21. Normal adult male. Ependyma (e) and subependymal tissue (s) of the wall of the third (III) ventricle.  $\times 110$ .

FIG. 22. Normal extracerebral (meningeal) artery. Branch of the arteria chorioidea. Human adult.  $\times 115$ .

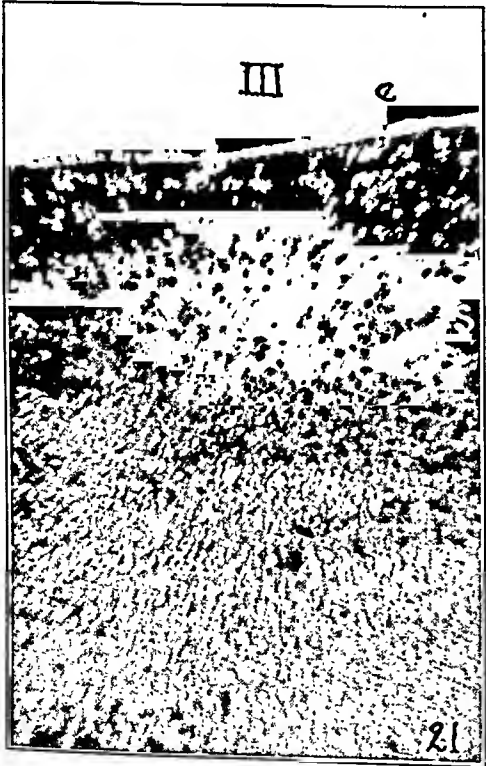
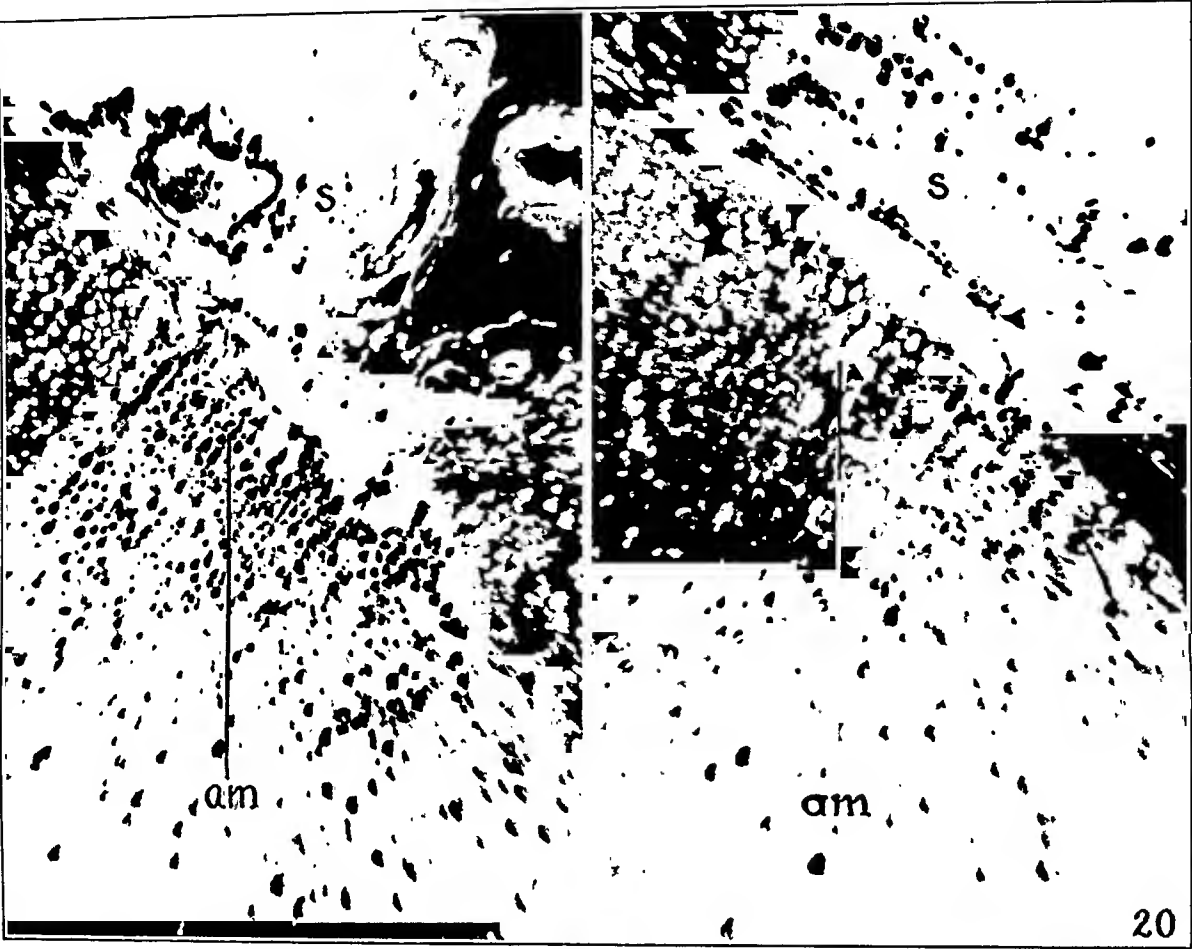




PLATE 70

FIG. 23. Granular ependymitis in hemorrhagic superior polioencephalitis (Wernicke's disease) from the wall of the third (III) ventricle in a case of incipient delirium tremens, chronic alcoholism and avitaminosis, complicated by cardiac decompensation and syphilis. Female, 40 years of age. e = ependyma; s = subependymal tissue.  $\times 110$ .

FIG. 24. Diseased extracerebral (meningeal) artery in cerebral arteriosclerosis from a 56 year old male. Branch of the arteria chorioidea. lb = lumen of blood vessel; ip = intimal proliferation; iem = inner elastic membrane; iemd = inner elastic membrane, delaminated; iemi = inner elastic membrane, inner lamina; m = media.  $\times 115$ .

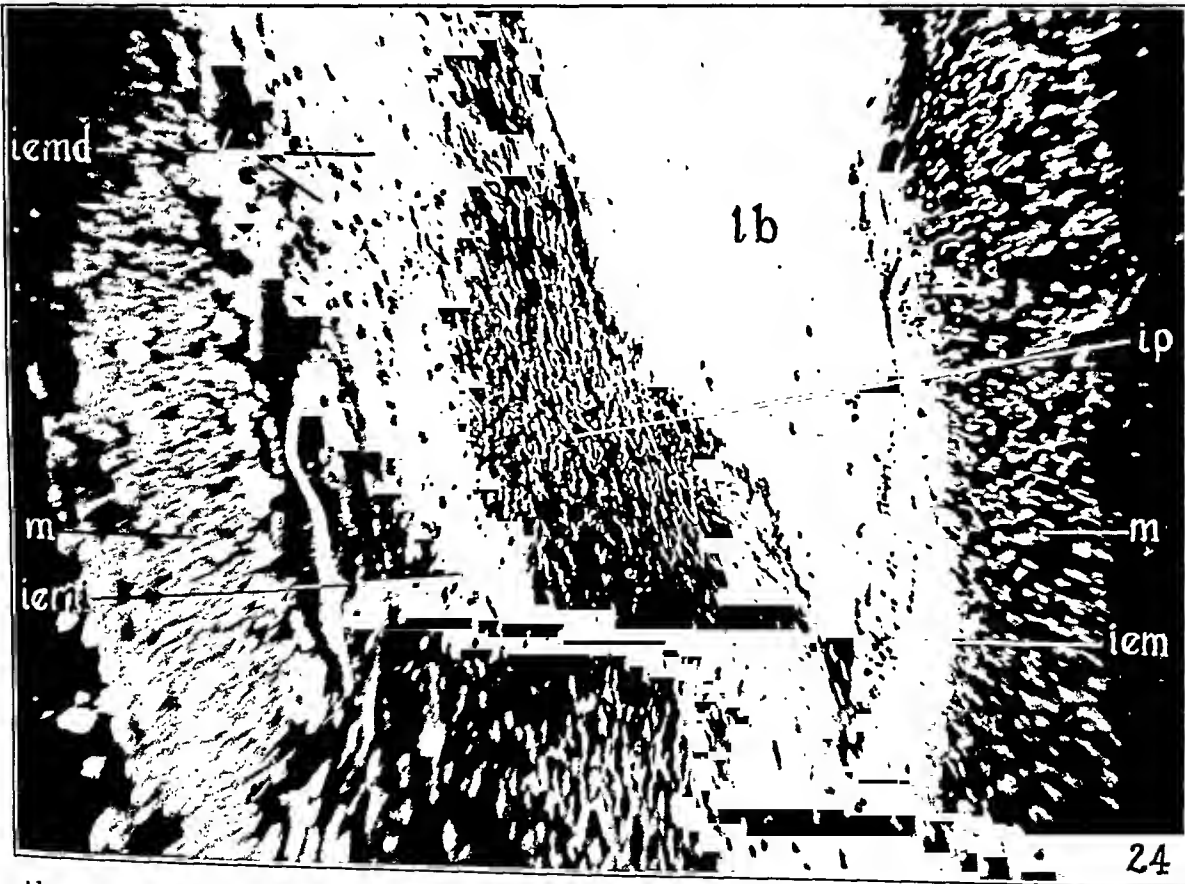
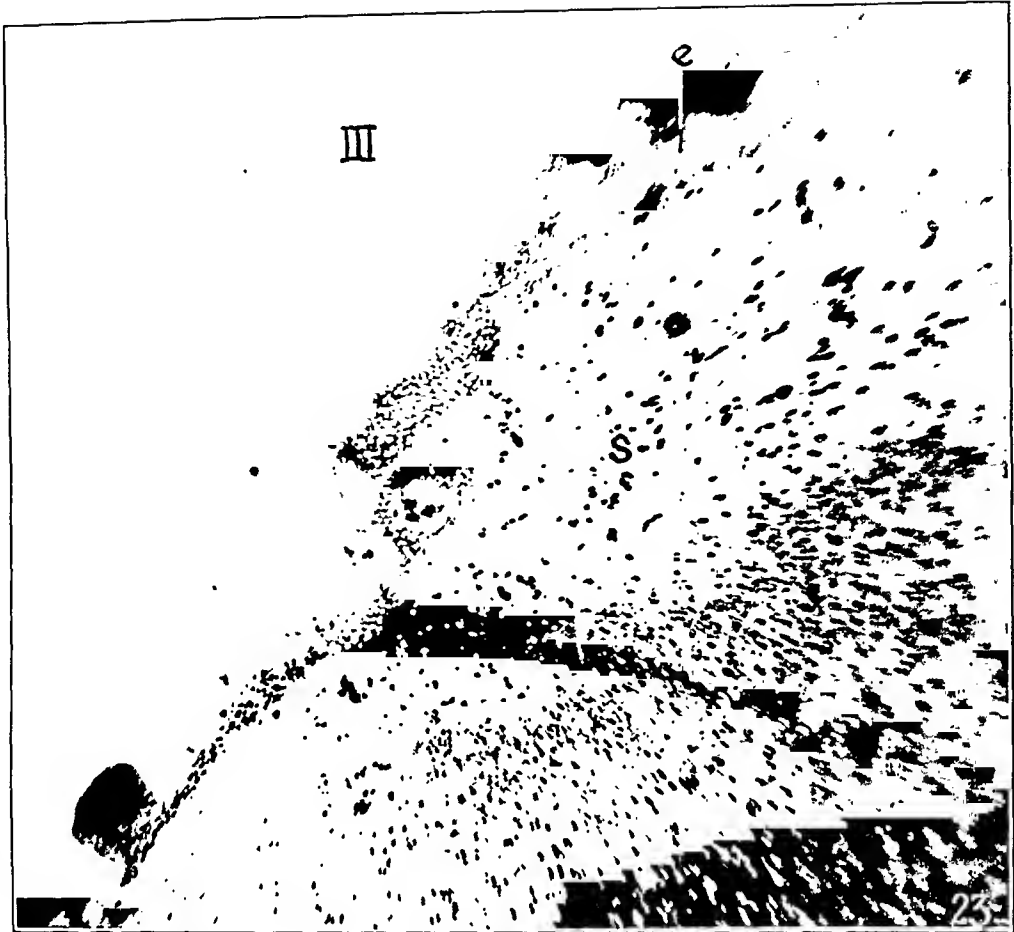
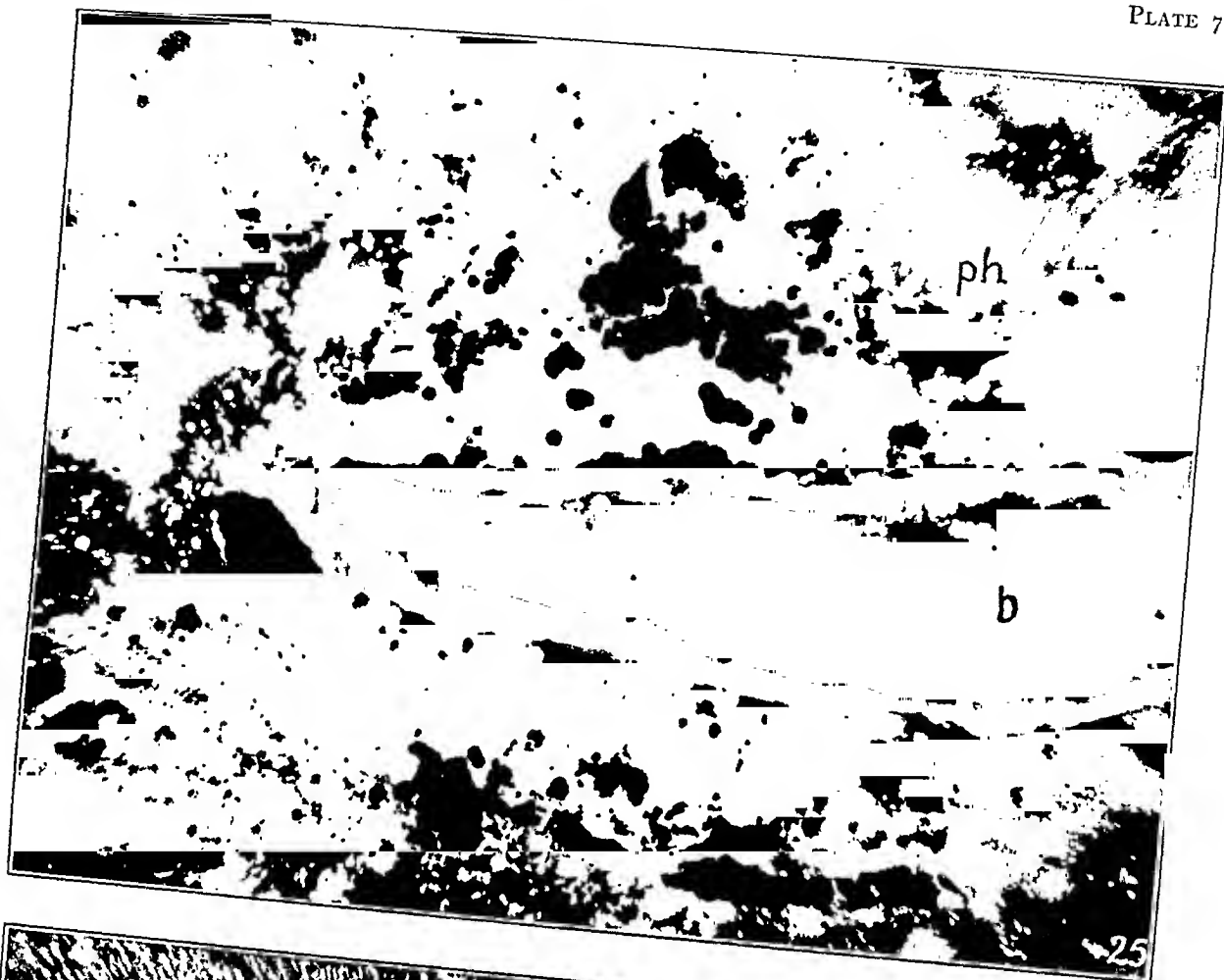


PLATE 71

FIG. 25. Perivascular hemorrhage. From the periventricular region of the hypothalamus in a case of superior hemorrhagic polioencephalitis in a 40 year old female. b = lumen of blood vessel; ph = perivascular hemorrhage. (From the same case illustrated in Fig. 23.)  $\times 500$ .

FIG. 26. Focus of softening in the central white matter of the brain. Cerebral arteriosclerosis in a 56 year old male. s = focus of softening; n = normal brain tissue.  $\times 100$ .



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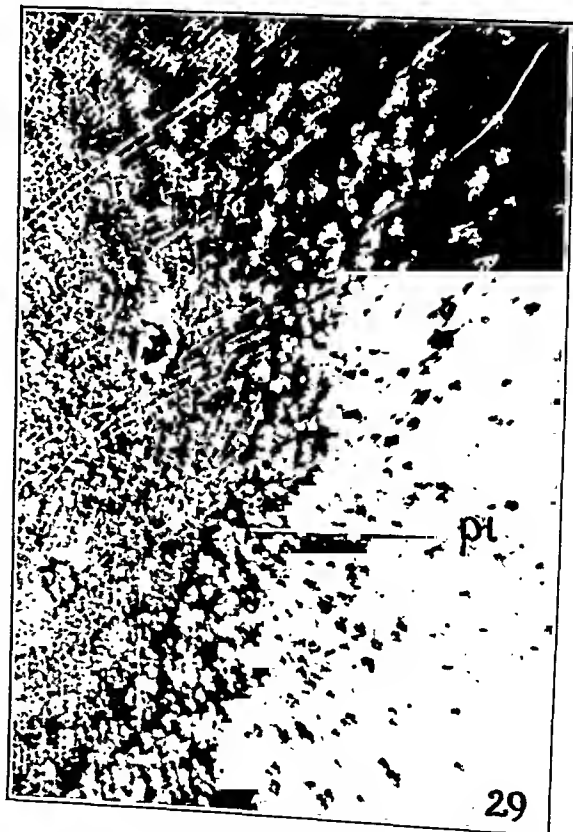
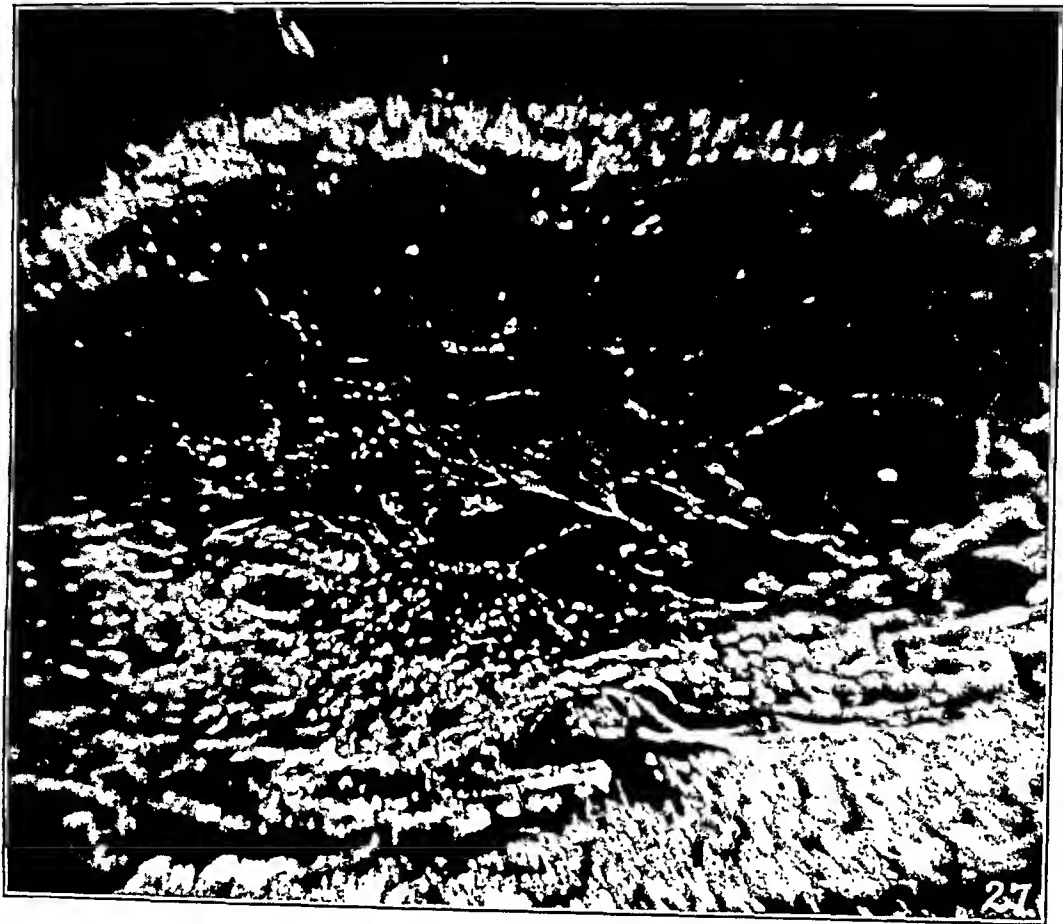
Mineral Content of Cerebral Lesions

PLATE 72

FIG. 27. Thrombosed extracerebral (meningeal) artery. Cerebral arteriosclerosis in a 56 year old male.  $\times 115$ .

FIG. 28. The purulent meningeal exudate (pe) covering the cerebral cortex (cc) in a case of pneumococcus meningitis. Male 49 years old.  $\times 115$ .

FIG. 29. Perivascular polymorphonuclear infiltration (pi) of an intracortical cerebral blood vessel in an area of purulent meningoencephalitis in a case of pneumococcus meningitis. Male 49 years old.  $\times 115$ .



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Mineral Content of Cerebral Lesions

PLATE 73

- FIG. 30. A metastatic tumor nodule in the brain (primary carcinoma of the bronchial epithelium with squamous metaplasia) from a 73 year old male. t = tumor; n = normal brain tissue.  $\times 115$ .
- FIG. 31. Tumor cells seen in Figure 30 (cerebral metastasis from primary carcinoma of the bronchial epithelium with squamous metaplasia) at higher power.  $\times 540$ .

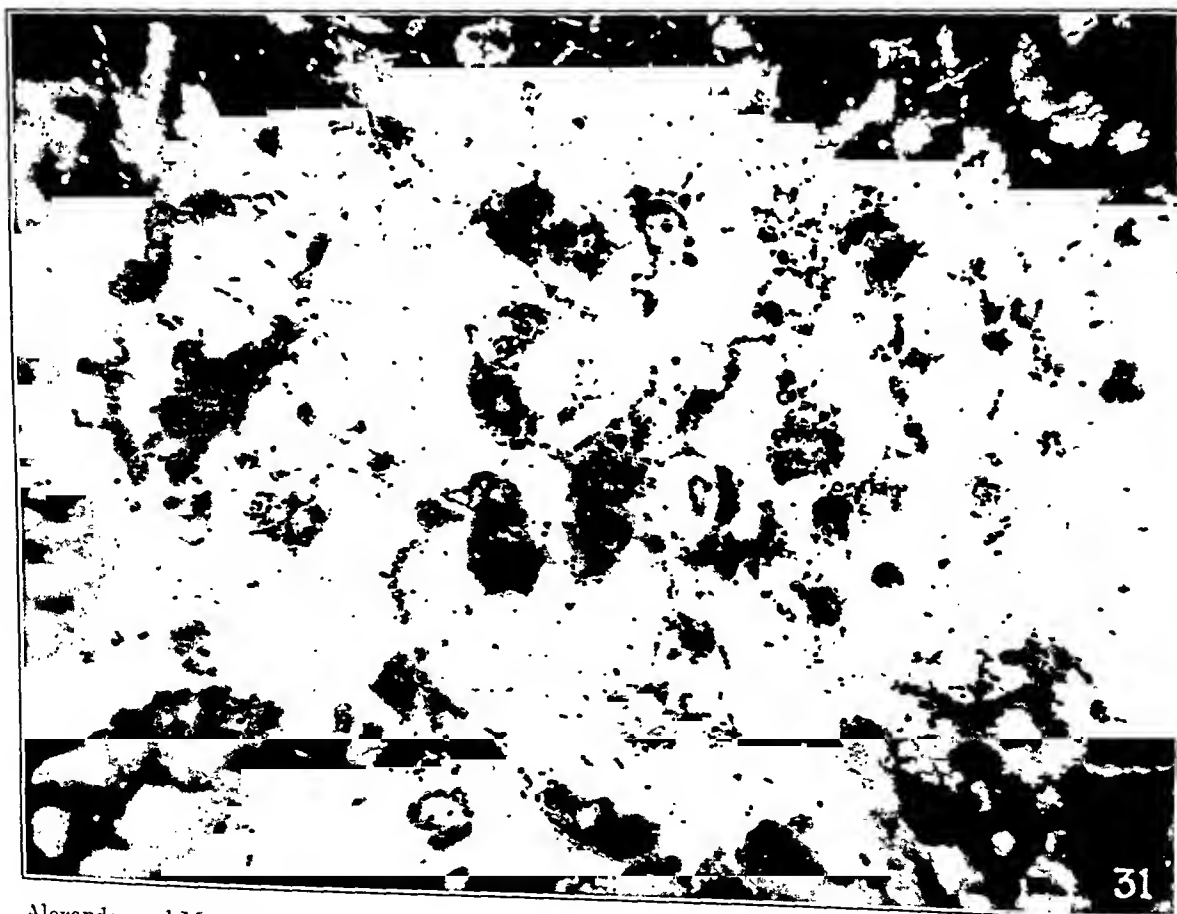
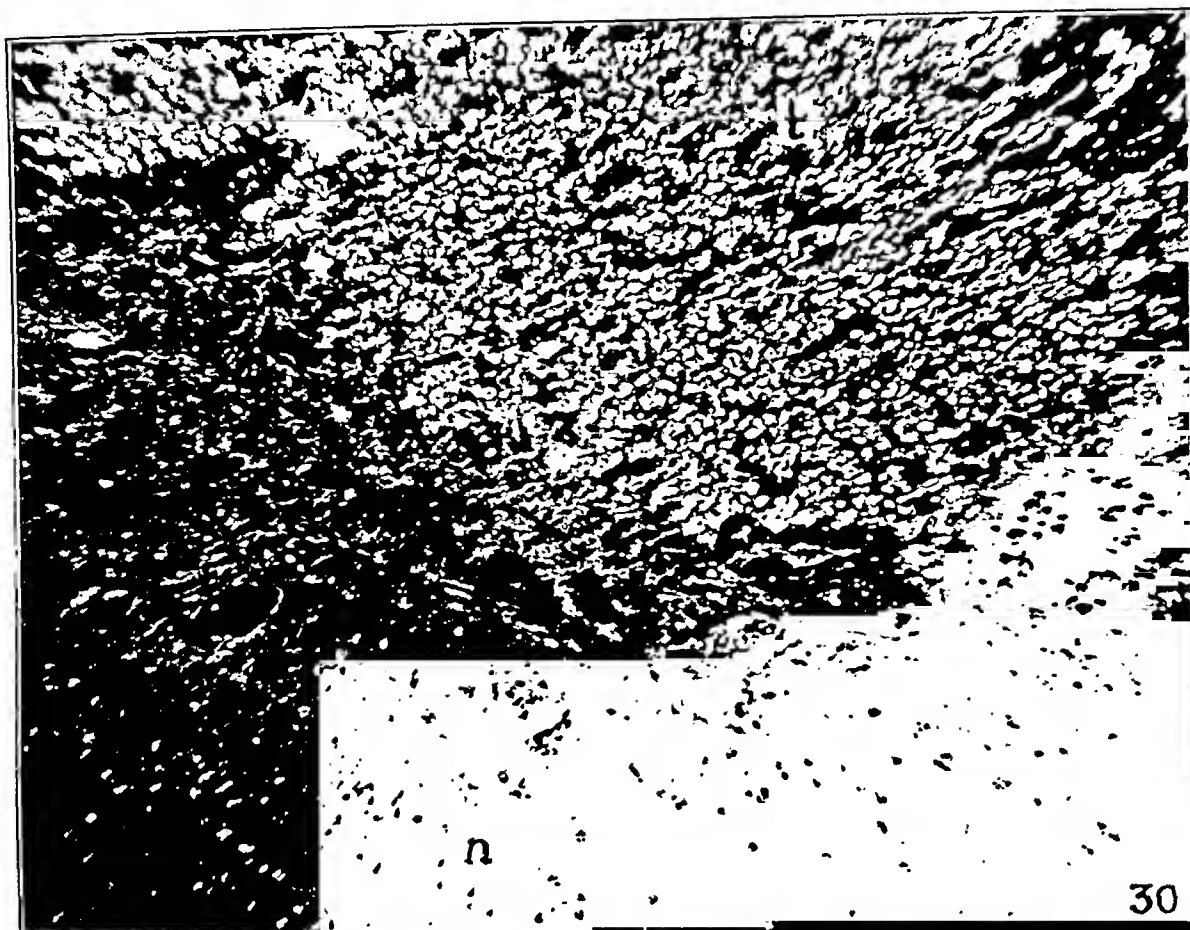




PLATE 74

FIG. 32. Normal blood smear, human adult.  $\times 530$ .

FIG. 33. A gumma (G) in the right substantia perforata anterior in a case of dementia paralytica. Male 45 years old. Oblique transillumination.  $\times 8$ .

FIG. 34. A sclerotic plaque in the subcortical white matter of the brain from a case of multiple sclerosis. s = sclerotic plaque; n = normal white matter; g = normal gray matter. Microincinerated preparation (frozen section)  $10\mu$  in thickness. Oblique transillumination.  $\times 7$ .

FIG. 35. From a sclerotic plaque in the subcortical white matter in a case of multiple sclerosis in a 44 year old female. s = sclerotic plaque; n = normal brain tissue.  $\times 110$ .

FIG. 36. From a sclerotic plaque in the subcortical white matter. Multiple sclerosis in a 44 year old female. s = sclerotic plaque; n = normal brain tissue.  $\times 110$ .

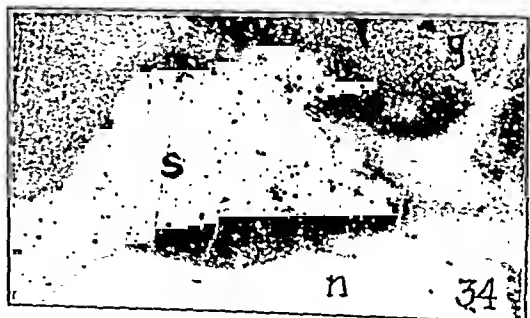
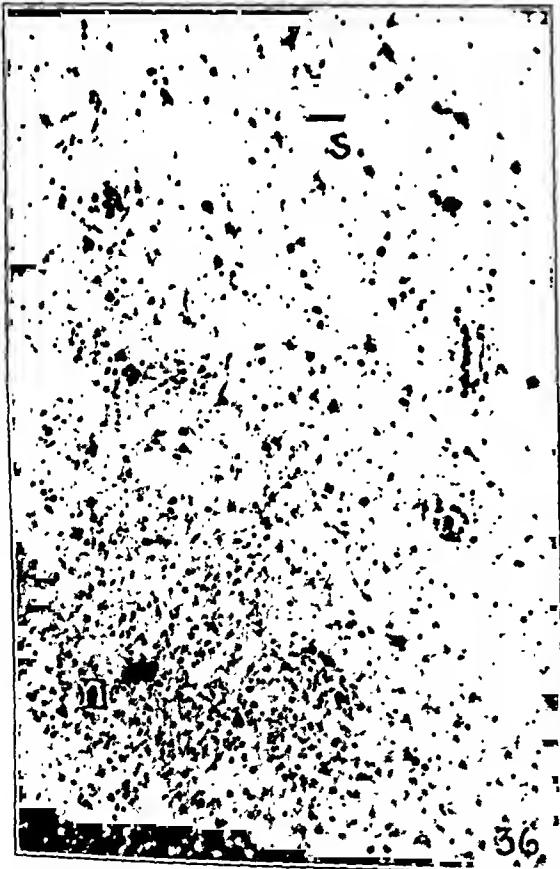
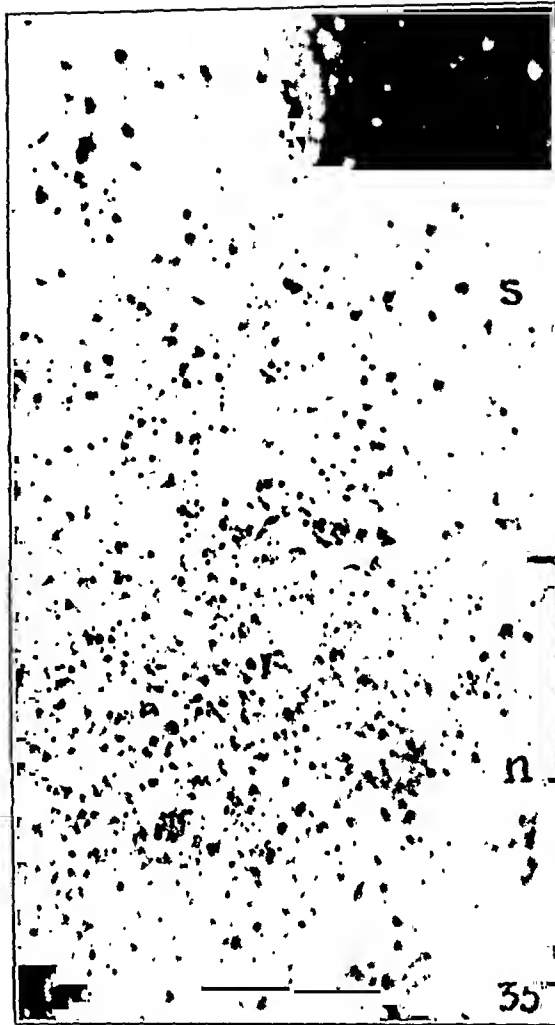
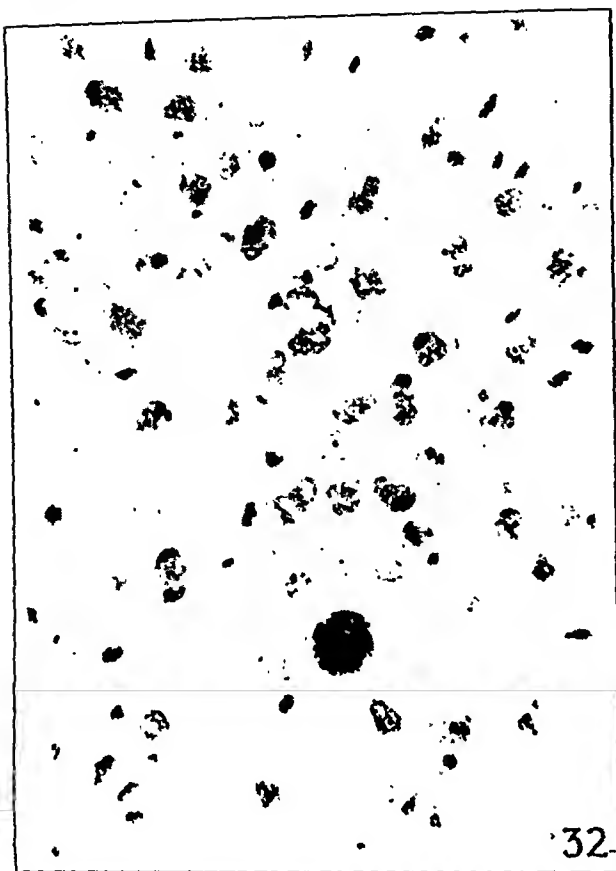
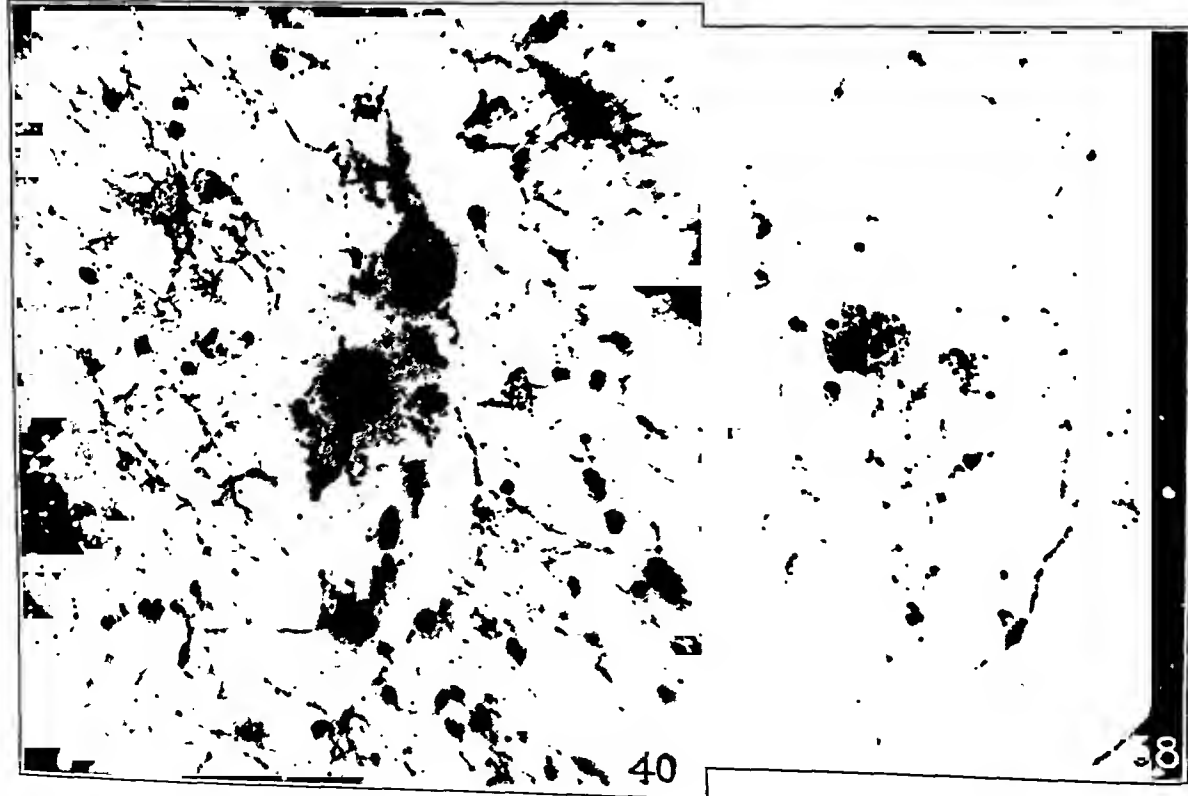
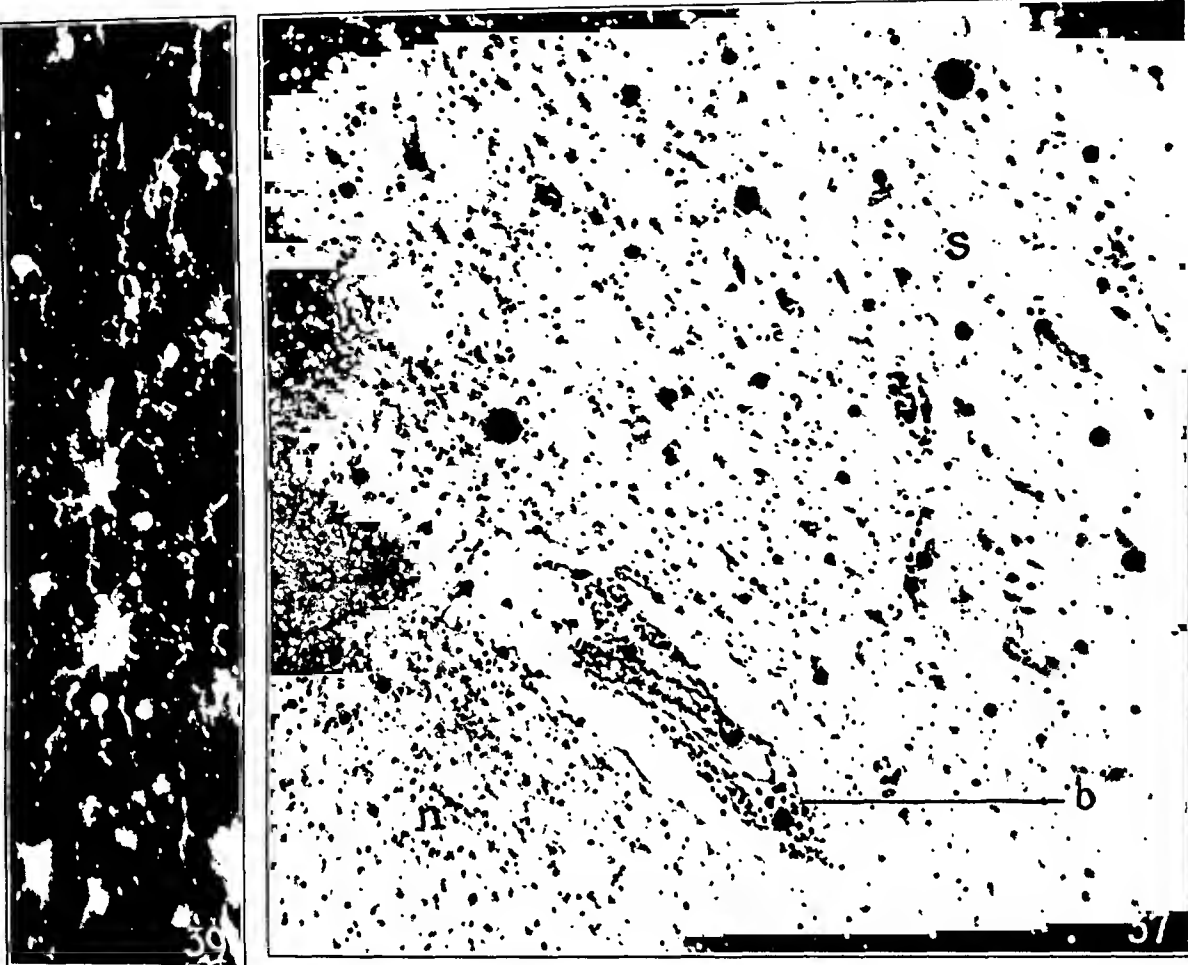


PLATE 75

FIG. 37. An early, hyperemic plaque in the subcortical white matter of the brain from a case of multiple sclerosis in a 44 year old female. s = sclerotic plaque; n = normal tissue; b = blood vessel on edge of plaque with perivascular infiltration consisting of lymphocytes and large phagocytic elements loaded with yellowish red mineral granules.  $\times 110$ .

FIG. 38. A large phagocytic histiocyte containing granules of iron oxide in the infiltrated adventitia of a blood vessel from an early subcortical plaque from a case of multiple sclerosis in a 44 year old female.  $\times 520$ .

FIGS. 39 and 40. Protoplasmic glial astrocytes in an early plaque in the subcortical white matter from a case of multiple sclerosis in a 44 year old female. In the center of Figure 40 two cross sections through a congested small vein are seen, with perivascular accumulation of lymphocytes and phagocytic histiocytes.  $\times 520$ .



## PLATE 76

- FIG. 41. An old anemic plaque in the subcortical white matter of the brain from a case of multiple sclerosis in a 50 year old female. Note the nuclei and processes of fibrillary astrocytes visible at low power.  $\times 110$ .
- FIG. 42. Fibrillary glial astrocyte in an old plaque in the subcortical white matter at higher power. From a case of multiple sclerosis in a 50 year old female.  $\times 520$ .
- FIG. 43. The cornu ammonis (field H 4 and the fascia dentata) in a case of Alzheimer's disease (focal senile atrophy) in a 74 year old female. Note three argentophilic senile plaques (S). Paraffin section stained by a modification of Bielschowsky's silver impregnation. Light field transillumination.  $\times 97$ .
- FIG. 44. Neighboring section of Figure 43. Note the absence of appreciable traces in the mineral picture at the sites corresponding to the senile plaques in Figure 43.  $\times 97$ .

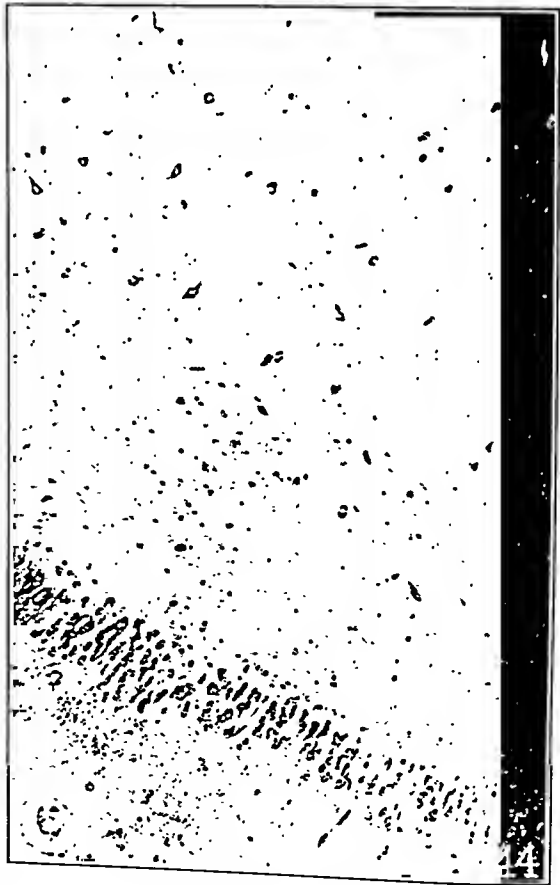
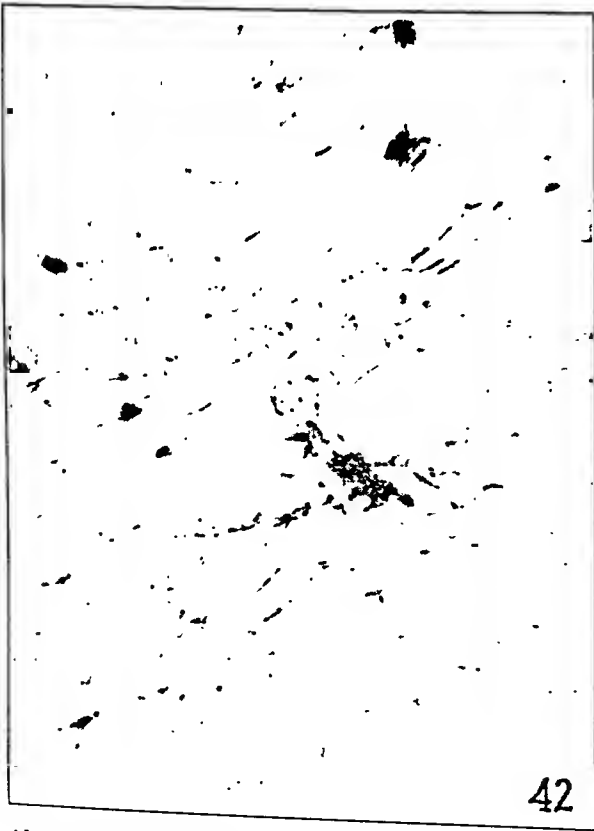
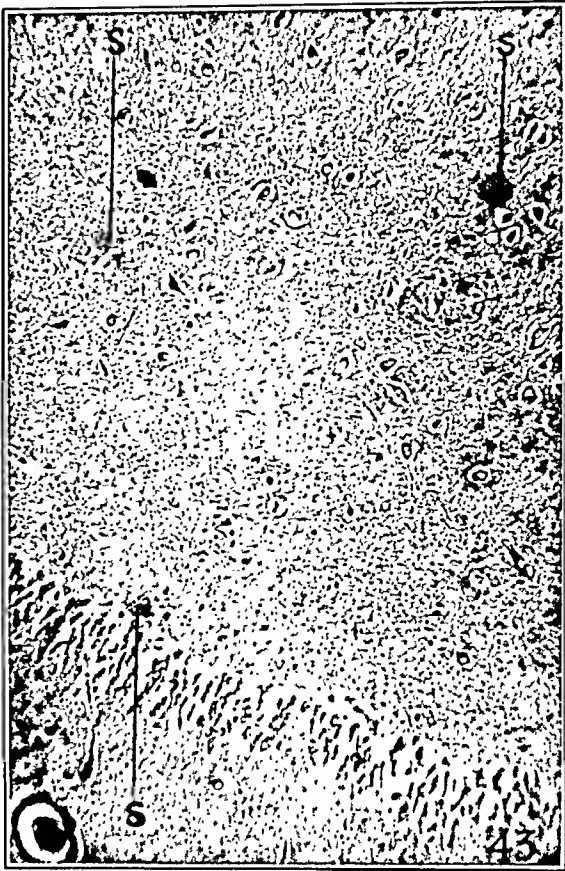
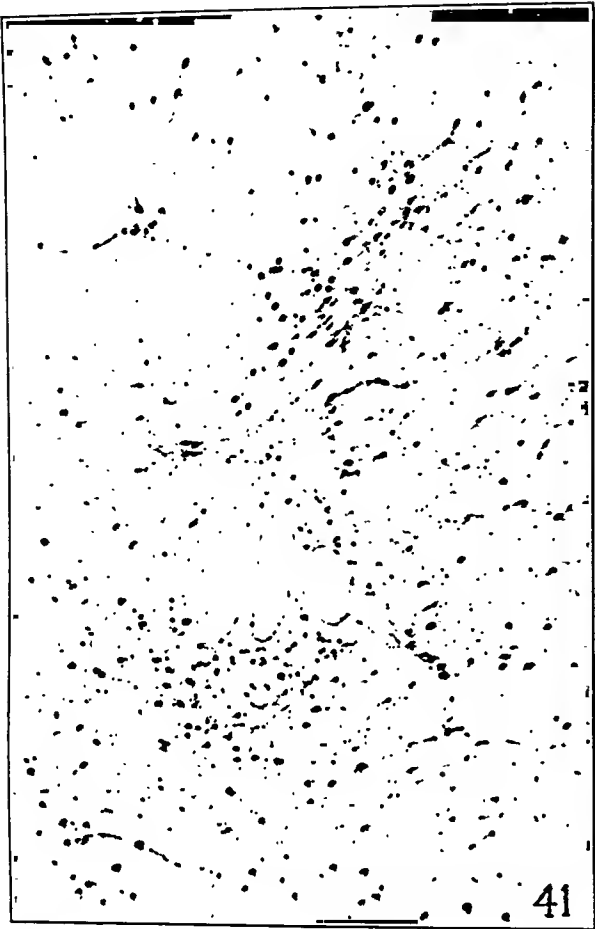


PLATE 77

FIG. 45. One of the senile plaques seen in Figure 43, dorsal to the fascia dentata, to the left of a precapillary blood vessel, (b) at higher power. s = senile plaque. From a case of Alzheimer's disease in a 74 year old female. Modification of Bielschowsky's silver impregnation. Light field transillumination.  $\times 514$ .

FIG. 46. Microscopic field corresponding to Figure 45 in a microincinerated neighboring section. The site corresponding to the senile plaque seen in Figure 45, dorsal to the fascia dentata, to the left of the same precapillary blood vessel (b) in this microincinerated preparation at higher power is marked only by a slight disarrangement of the glial reticulum, however, not by demineralization or by excess mineral residue.  $\times 514$ .

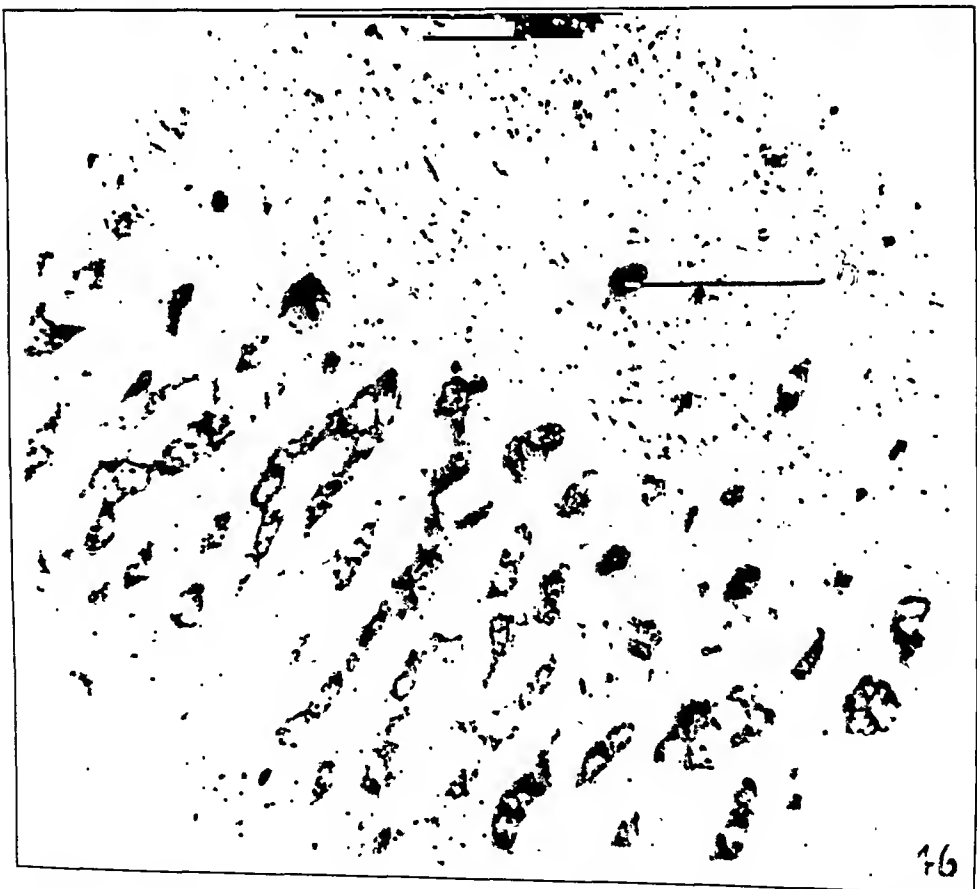
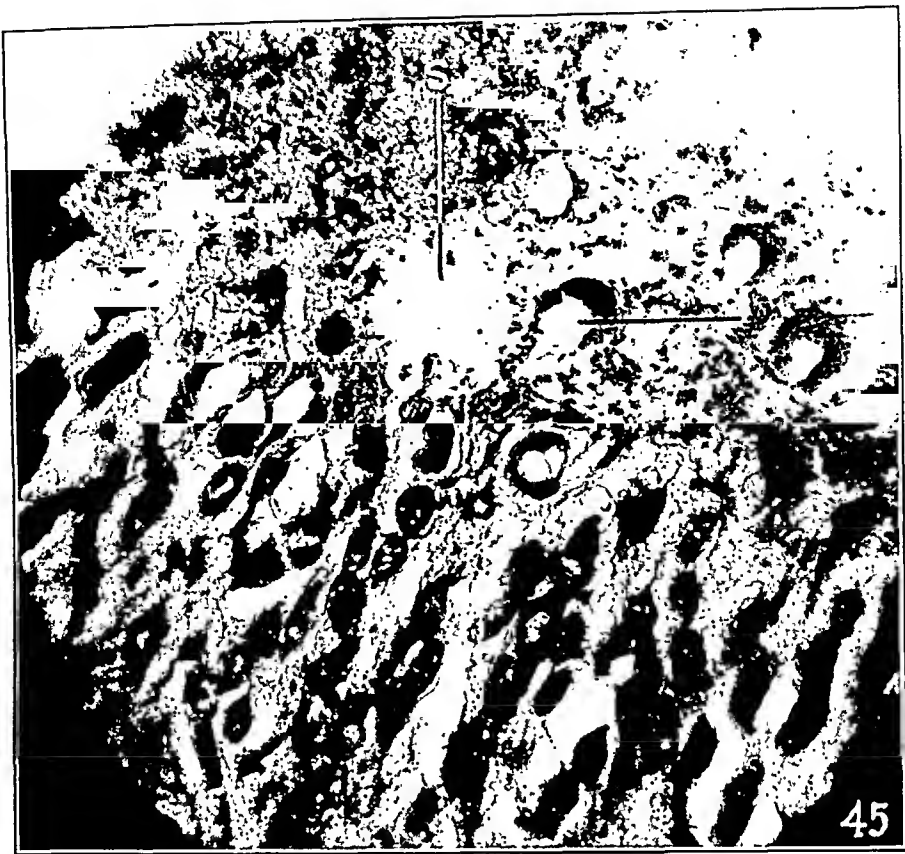
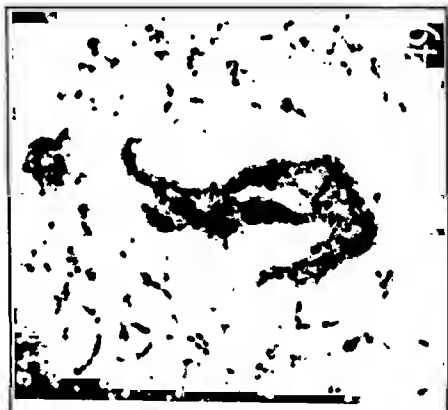




PLATE 78

- FIG. 47. The area entorhinalis in Pick's disease (focal senile atrophy), in a 72 year old female. *l* = lamina zonalis; Pre-*Alpha* = lamina principalis externa, sublayer *Alpha*; Pre-*Beta* and *Gamma* = lamina principalis externa, sublayers *Beta* and *Gamma*; *Ds* = lamina dissecans; *Pri* = lamina principalis interna.  $\times 110$ .
- FIG. 48. A pyknotic (shrunken) ganglion cell and a glial rosette from the cortex of the subiculum cornu ammonis in a case of Alzheimer's focal senile dementia in a 74 year old female.  $\times 1000$ .
- FIG. 49. Alzheimer's ganglion cell disease in a ganglion cell of pyramidal type from the cortex of the subiculum cornu ammonis. Alzheimer's disease (focal senile dementia) in a 74 year old female. The characteristic thickened, winding neurofibrillar strands stand out by their substantial mineral content, while the remainder of the cytoplasm of the cell is demineralized.  $\times 1500$ .
- FIG. 50. Three ganglion cells from the cortex of the subiculum cornu ammonis in a case of Alzheimer's disease in a 74 year old female.  $\times 1000$ .





# STUDIES ON THE EXPERIMENTAL INFECTION OF SOME REPTILES, AMPHIBIA AND FISH WITH *SERRATIA ANOLIUM* \*

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In a preceding paper, Duran-Reynals and Clausen<sup>1</sup> (1936), a microorganism was described as the causative agent of a tumor-like disease found in a species of Cuban lizard, *Anolis equestris*. The organism is a motile, Gram-negative rod, producing a pink pigment under certain conditions. It has been temporarily classified as belonging to the family *Bacteriaceae*, tribe *Chromobacterieae*, genus *Serratia*. This organism was described in a previous paper as *Serratia anolium*.

In this investigation we have studied the pathogenic effects of *S. anolium* on many cold-blooded animals, including the species in which the disease was originally discovered. A point of general interest, namely the action of temperature on infection, which affects many species of cold-blooded animals, was especially fitted for such studies.

## MATERIAL AND METHODS

The animals used in the following experiments were as follows: The iguanid lizards, *Anolis equestris* and *Anolis carolinensis*; the gekkonid lizards, *Tarentola mauritanica* and *Hemidactylus brookii*; the snakes, *Thamnophis butleri* and *Storeria dekayi*; the musk turtle, *Sternotherus odoratus*; the toad, *Bufo americanus*; the frog, *Rana pipiens*; and the catfish, *Ameiurus melas*.

All of the above animals used for inoculation tests were selected from the stock animals which had been kept in the laboratory for some time and hence had become adjusted to laboratory conditions. Healthy specimens of *A. equestris* were also secured from Cuba during the course of the experiment.† They were kept under observation

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† We are greatly indebted to Dr. F. Ramirez-Corria of the Findlay Institute of Havana for sending these lizards.

for several days before inoculation. The experimental animals were placed in individual cages or aquaria and food and water were kept in the containers at all times. The food consisted of a supply of larvae of the meal beetle (*Tenebrio molitor*) and the wax moth (*Galleria mellonella*). Earthworms were also placed in the cages or aquaria containing the snakes or fish.

Twenty-four hour cultures in broth or agar, suspended in 10 cc. of saline, were used for the inoculations. In a few cases the animals were directly grafted with tumor-like masses.

Further details regarding specific methods will be included in the respective sections of the results to follow.

## EXPERIMENTAL

### *Inoculation of Anolis equestris*

In different experiments lasting several months 6 *A. equestris* were injected subcutaneously in the hind limb with a 0.2 cc. suspension of agar culture. Swelling developed in all animals within 2 to 3 days after the injection. This swelling increased considerably during the following week. In some cases the infection localized in the knee joint and resulted in the formation of a voluminous round mass (Fig. 1). This mass persisted for several months and gradually disappeared. In 3 other cases the swelling was more diffuse (Fig. 2) and the animals died within 6 to 7 weeks after the injection. Post-mortem examination of 2 of the lizards showed degeneration of the tissues which exhibited a yellowish color and were surrounded by an area of hemorrhage. The causative organism was readily recovered from the injected site, and from the heart blood as well. In the 3rd lizard postmortem examination showed that the muscle of the injected limb was "shot" with small nodules. Large nodules with necrotic centers were found in the deeper layers. A small mass of hard opalescent tissue with a necrotic center was also found beneath the angle of the jaw. The liver was also "peppered" with pinhead whitish areas. The organism was recovered from the hind limb, the nodule in the jaw region, the liver and also from the peritoneal cavity. In another *A. equestris*, similarly injected, a chronic process developed with the formation of a nodule persisting for several weeks. Finally, in another test 1 animal was injected in the splanchnic cavity without any resulting ill effect. Of the above mentioned

animals, 4 were additionally inoculated by scarification of toes and vocal pouch. No infection resulted after such treatment.

It may be concluded from these tests that cultures of *S. anolium* injected into *A. equestris* result in the production of a condition which, in its chronicity and localized character, resembles the natural disease of the same animal.

### *Effect of Environmental Temperature on the Experimental Disease in Anolis equestris*

Dealing with an infection of cold-blooded animals it was obviously interesting to study the modification of the infection when the temperature was elevated. Accordingly, some tests were carried out on *A. equestris* in which the action of *S. anolium* was studied with higher temperatures.

In one experiment 2 lizards were injected subcutaneously with 0.5 cc. of saline suspension of a 24 hour agar culture. Immediately afterwards 1 of the animals was placed in the incubator at 37° C. Swelling developed in both animals during the first several days, but at the end of the 3rd day the swelling had practically disappeared in the animal kept at 37° C., whereas it steadily increased in the control animal left at room temperature. The animal at 37° C. lost considerable weight and died on the 5th day. By that time the animal kept at room temperature showed a marked inflammation of the entire thigh. It was then placed in the 37° C. compartment. In the course of the next 2 days the inflammation subsided and was limited to a small region in the anterior portion of the thigh. The animal appeared sick, lost weight and died on the 7th day after being placed at 37° C. The bacterium was abundantly found in the injected thigh and the blood.

In a second experiment 2 more *Anolis* were injected as described above. Ten days later both animals showed a slight swelling in the injected region. One of them was placed at 37° C. and the other was left as a control at room temperature. In the former lizard the inflammation subsided; the animal was taken sick and died 7 days after being placed at 37° C. The causative bacteria were also isolated from the injected region and from the heart. The control animal at room temperature developed a lesion in the knee which lasted several months and finally disappeared, leaving a permanent deformity of the bones.

As a further control to these experiments a non-injected *A. equestris* was left at 37° C. for several days. No alteration in its health was apparent.

*Effect of Staphylococcus aureus, Streptococcus hemolyticus and Meningococcus on Anolis equestris*

It was desirable to know whether the experimental lesions obtained in *A. equestris* by *S. anolium* were of a specific type or could be reproduced by agents pathogenic for mammals, such as staphylococcus, streptococcus and meningococcus. Accordingly, the *A. equestris* were each injected under the skin of the thigh with 0.2 cc. of saline suspension of a 24 hour agar or blood agar culture of the above mentioned bacteria. In the case of meningococcus, mucin was added to the inoculum in order to enhance the infective power of the bacteria. After injections were made the corresponding bacteria were seeded in adequate media and left at room temperature, as were the injected lizards. In each case a growth was obtained. The results of these tests may be summarized as follows. A localized inflammation without any specific character resulted immediately after the injection. This remained stationary for a few days and had practically disappeared after 2 weeks. No general ill effect could be noticed.

In another test 1 lizard was injected with meningococcus mixed with mucin and left at 37° C. for 2 weeks. The result was identical with that shown when the animal was kept at room temperature.

Thus, 3 bacterial species of marked pathogenic power for mammals proved to be devoid of any serious infective power for *A. equestris*.

*Anolis carolinensis*

After having reproduced the disease in the species which originally showed it, it was thought desirable to study the susceptibility of other cold-blooded animals. A species of lizard related to *A. equestris*, namely *A. carolinensis*, was first used.

In the first experiment 5 *A. carolinensis* were directly grafted under the skin of the thigh or the foreleg with small portions of the healthy viscid tissue of a nodule recently removed from an *A. equestris*. All the animals developed an acute condition in the injected region and 2 of them died 10 and 23 days, respectively, after the in-

jection. Locally, an abscess was formed from which the organism was recovered. It was also recovered from the blood. In the other lizards a localized and raised tumor-like condition developed. This condition either persisted for several weeks without rupturing or a suppurative process was established which lasted for an extended period. Lesions healed after 2 to 3 months, leaving a hard nodule.

TABLE I

*Results of Various Concentrations of Inoculum from 24 Hour Cultures of Serratia anolium on the Lizard, Anolis carolinensis*

No. of cases	Dilutions *	Lesions	Cultures †
	cc.		
2	0.02	Positive	From nodules & heart
2	0.005	Positive	From nodules
2	0.002	Negative	From heart in 1 case
3	0.001	Negative	From heart in 1 case
2	0.0001	Negative	Negative

\* Dilutions were made from 24 hour cultures in broth or agar, suspended in 10 cc. of saline.

† Animals were sacrificed 3 months after injection and cultures and smears were made at that time.

In another test 2 *A. carolinensis*, which were left from the initial experiment and which still exhibited nodular lesions in the foreleg, were injected in the opposite foreleg with 0.1 cc. of a 24 hour broth culture of the bacterium. As a control, 2 normal lizards of the same species were similarly injected. All the lizards died between the 3rd and 6th day after inoculation. The causative bacterium was recovered from the local lesion and from the blood of the entire group of animals.

In a third series of experiments it was thought of interest to determine, if possible, the concentration of the inoculated medium necessary to produce the typical lesions. Hence, a series of concentrations was made. Dilutions were all made from 24 hour cultures suspended in 10 cc. of saline. Each animal was injected in the thigh of the hind limb with 0.1 cc. of the suspension. The injected lizards were kept in one cage at 24° C. with 5 normal non-injected animals.

The results, as recorded in Table I, show that, up to a certain point, the localization and nodule formation were dependent on the dilution and that as the concentration decreased beyond this point the lesions failed to appear. Nevertheless, the latter low concentrations often remained virulent and were found in the heart blood



for a considerable period even though no local lesions occurred. The normal lizards, on postmortem examination, revealed no evidence of the causative bacterial organism.

### *Inoculation of Gekkonid Lizards*

Since experiments on *A. carolinensis* had shown that the bacterial organism *Serratia anolium* would produce pathogenic effects comparable to those produced in another species of lizard of the same genus, it was of importance to repeat the inoculations in an entirely different group of lizards.

Three *Tarentola mauritanica* lizards were injected subcutaneously in the left hind limb with 0.2 cc. of the suspension from a 24 hour culture. Two of the lizards were kept at room temperature and the other was placed at 37° C. The 2 former showed a slight swelling in the injected area 10 days after the injections and lesions developed in the injected limbs. These lesions continued to enlarge during the month following (Fig. 3) and the bacterium was recovered from the lesions and also from the heart blood 2 months following injection. The increase in size of the limbs had apparently subsided at this time but no decrease in size could be noted. The lizard at 37° C. died 11 days after the injection and the causative organism was isolated from the injected region, the peritoneal cavity and the heart blood. These experiments were then repeated on another gekkonid lizard, *Hemidactylus brookii*, and the results were similar to those obtained in *T. mauritanica*.

### *Experiments with Turtles*

Since the bacterium *S. anolium* produced tumor-like lesions in several species of lizards, it was thought necessary to repeat some of the above tests on other reptilian forms. Hence, the musk turtles (*Sternotherus odoratus*) were selected. Two turtles were injected with 0.2 cc. of the inoculum into the right hind limb and into the foot of the left forelimb, respectively. No swelling or nodule formation was noticeable for 2 months in either of the turtles. After 3 months the region of injection showed rapid signs of enlargement. These nodules increased in size very rapidly during the following month (Fig. 4), and at the end of the 4th month both turtles were sacrificed. The causative bacterium was easily isolated from the lesions as well as from the heart blood in all instances.

### *Experiments with Snakes*

The garter snakes, *Thamnophis butleri*, and the brown snakes, *Storeria dekayi*, were selected for injection to see if the bacterium, *S. anolium*, would also produce tumor-like lesions in this group of reptiles. Accordingly, 3 *T. butleri* were injected, subcutaneously, about 3 cm. caudal to the head region. Two snakes were left at 24° C., while the 3rd was placed at 37° C.

In the case of the 2 snakes at 24° C., the nodules began to form 3 weeks after injection and reached their maximum size at 6 weeks (Fig. 5). After 6 weeks the nodules failed to increase in size but continued to subsist for a period of 4 months. The bacterium *S. anolium* was recovered from the lesion and from the heart blood in both cases. In the snake at 37° C. slight swelling was evident 3 days following injection but the animal died 9 days after injection. The causative bacterium was recovered from both the lesion and the heart blood.

These experiments were then repeated on another species of snake (*Storeria dekayi*) and exactly the same results were obtained.

### *Experiments on Amphibians*

Two frogs, *Rana pipiens*, and 2 toads, *Bufo americanus*, were injected with 0.2 cc. into the hind limb. At the end of 4 days a noticeable swelling was evident in both species and at the end of 3 weeks the injected portion of the thigh had increased three times its normal size. In 1 of the toads the integument over the enlarged nodule was broken. In this latter case a mixed culture was obtained but the bacterium *S. anolium* was included. In the case of the 2 frogs and the remaining toad the animals were sacrificed 1 month following injection. Cultures of the bacterium were obtained from the lesions in all 3 instances. The heart blood also contained the causative organisms in all 3 cases. In 1 frog the tibia bone was broken (Fig. 6) and microscopic sections showed a degeneration of the tissue with the bacterium invading the marrow of the bone as well as the surrounding tissues.

### *Injection Experiments on Fish*

Three small catfish, *Ameiurus melas*, were selected from a group of 6 which had been brought into the laboratory 2 years before, and

injected with 0.1 cc. of the inoculum. Injections were made on the left side approximately equidistant from the head and caudal fin. The 3 injected fish were placed in the same aquarium with 3 non-injected catfish. At the end of 3 days the area of injection became quite necrotic and the integument and underlying tissues sloughed (Fig. 6). Ten days later this area had become completely healed and the fish showed no lesions and appeared normal in all respects. At the end of 3 months all the fish were sacrificed and the bacterium was recovered from the heart blood of not only the injected ones but 2 of the 3 non-injected ones as well.

These results show that the bacterial organism in question is retained in the fish for at least 3 months and, when cultured, shows all of the growth characteristics of the bacterium from other cold-blooded forms tested even though lesions were never produced in this species of fish. It was also of interest to show that the organism could be passed through the medium of the water to a non-injected specimen.

### *Tests with the Rabbit, Guinea Pig and Mouse*

After having studied the effects of *S. anolium* on cold-blooded animals it was obviously interesting to observe the effects it might have on some of the mammals commonly used in laboratory research.

Two rabbits were injected intracutaneously with 0.5 cc. of a 24 hour culture and a 3rd rabbit with 0.2 cc. of the same culture intracutaneously. The 2 former rabbits developed a necrotic lesion 3.5 by 5.5 cm., which was well localized and healed in 10 to 15 days. The latter animal did not show any symptoms of the disease whatsoever.

One guinea pig was injected in the skin and another in the peritoneum with 0.2 cc. of the same culture. In the former animal a round, slightly necrotic lesion, about 4 cm. in diameter, developed. This healed in approximately 10 days. The latter animal did not show any appreciable sign of the disease.

Ten mice were injected with doses ranging from 0.1 to 0.0001 cc. in the leg muscle. A slight swelling and redness developed in only 1 animal, the animal injected with the largest quantity. This reaction, however, disappeared after 5 days. In another experiment 2 mice were injected intracutaneously with 0.1 and 0.2 cc., respectively, of a 24 hour broth culture. The mouse injected with 0.2

cc. died with an acute inflammation and necrosis of the abdominal tissues. The other mouse showed no apparent sign of the disease.

It can be concluded from these experiments that, as contrasted with the relatively high pathogenicity produced by *S. anolium* in amphibians, reptiles and to some extent fish, it is little or non-virulent for the mammals so far tested.

## DISCUSSION

Experimental tests have designated that the increase of temperature of a cold-blooded animal to an approximate level usually shown by mammals brings about a considerable modification in respect to this bacterial infection. What, under normal surrounding temperature ( $18^{\circ}$ – $24^{\circ}$  C.) is generally a chronic, well localized process, which rarely generalizes, becomes a general disease which results in the death of the animal in a few days if the temperature is increased to  $37^{\circ}$  C. At room temperature death was never observed prior to 6 weeks after injection. Co-existing with the generalization there is a considerable improvement and often times complete disappearance of the local lesion. It is possible that the local gain might be obtained at the expense of the invasion of the animal tissues by the bacterium.

On one hand, this series of experiments offers an analogy with, and might be considered the counterpart of, the classic experiment by Pasteur, who succeeded in infecting chickens, a naturally refractory bird, with anthrax by lowering the temperature of the bird after the experimental inoculation with *B. anthracis*.

Considered from another angle, the described results may be compared with experiments reported by Wollman and Uribe<sup>2</sup> (1931). These investigators observed that no antibodies against red cells and bacteria could be produced when these antigens were injected into a frog, *Calyptocephalus gayi*, kept at normal temperature. However, they obtained rather high titers when the temperature of the animals was increased. Other investigators, Dieudonné<sup>3</sup> (1894), Schire and Greenfield<sup>4</sup> (1929), and others, have also shown the differential bacterial reactions in cold-blooded forms when temperatures were altered and have also compared their effects on various warm-blooded animals.

During the course of this series of experiments a number of iguanid lizards, *Basiliscus vittatus*, were sent to the Museum labora-

tory from southern Mexico. Upon their arrival, a tumor-like lesion was noted on the hind limb of one of the animals. Cultures were made and the causative organism isolated. This bacterium was found to be the same species (*Serratia anolium*) as found in *Anolis equestris*. These findings are of special interest in that they serve to illustrate the more extensive distribution of this organism in nature.

The results as presented in this series of experiments should serve as additional stimulation toward speculation concerning infection and resistance in higher animals, and also to emphasize the importance which comparative immunology would have for a better understanding of these processes.

### SUMMARY

1. *Serratia anolium*, a bacterium which induces tumor-like lesions in the iguanid lizard *Anolis equestris*, was injected into other cold-blooded animals as well as several species of warm-blooded forms.

2. In all the forms of reptiles or amphibians used the lesions were produced and appeared to be similar in all respects to the natural disease. The causative bacterium was easily isolated and cultured from the lesion as well as from the heart blood.

3. In the experimentally injected cold-blooded forms which were kept at room temperatures, the bacterium, while being found in the blood, usually became localized and produced lesions. Diversely, the higher temperature (37° C.) for the same species of experimental animals, always produced general pathogenic effects and proved fatal in most instances.

4. In the warm-blooded animals used, the bacterium was non-virulent and no pathogenic effects were noted in any case.

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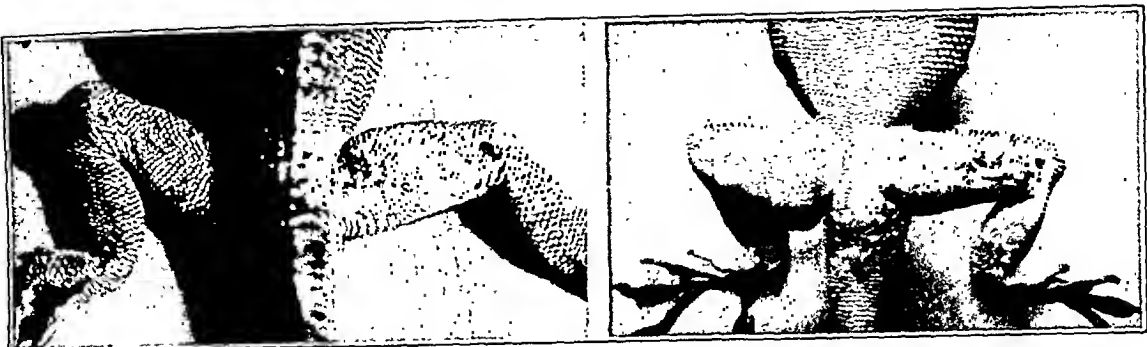
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## DESCRIPTION OF PLATE

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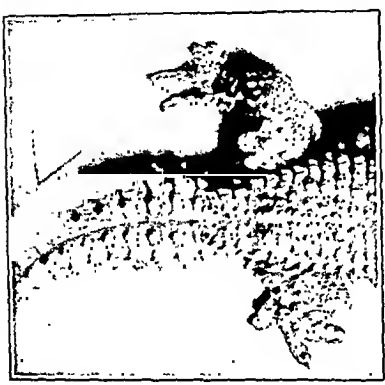
### PLATE 79

- FIG. 1. *Anolis equestris* injected with *Serratia anolium* in left thigh. Infection localized in the knee joint. Note voluminous round mass.
- FIG. 2. *A. equestris* injected with the bacterium in left thigh. Swelling most diffuse but localized. This size remained as shown for 7 weeks until the animal died.
- FIG. 3. *Tarentola mauritanica* injected subcutaneously in left hind limb 4 weeks after inoculation.
- FIG. 4. Ventral view of musk turtle showing nodule formation in right hind limb 4 months after inoculation.
- FIG. 5. The garter snake, *Thamnophis butleri*, showing nodule formation on the right side 6 weeks after inoculation.
- FIG. 6. Injected hind limb of leopard frog dissected to show the broken tibia extending from the center of the mass to the extreme left.
- FIG. 7. Inoculated catfish showing necrotic area in mid anterior-posterior region 3 days after injection of bacterium.



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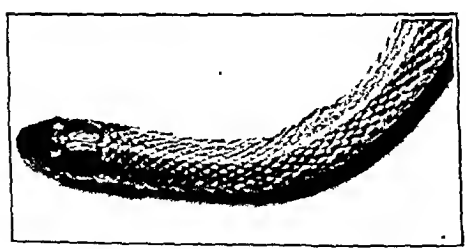
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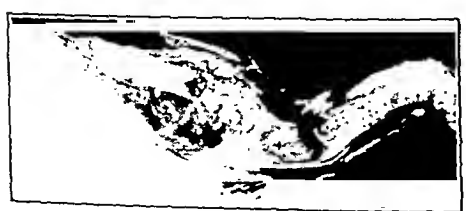
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6

Clausen and Duran-Reynals

Experimental Infection with *Serratia Anolium*





# STRUCTURE OF THE SMALL CEREBRAL ARTERIES AND THEIR CHANGES WITH AGE \*

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Many histologists believe that the arteries of the brain are anatomically different from those in other parts of the body; others feel that they are essentially the same. In reviewing the literature on the structure of normal cerebral vessels one is impressed by the paucity of reports. It was for this reason that our attention was directed to the investigation of the histology of the small cerebral vessels and the changes that occur in their walls with advancing age.

Triepel<sup>1</sup> in 1897 first described in detail the histological structure of the cerebral vessels. He suggested that they differed from similar sized vessels elsewhere in the body in three features: in the great prominence of the internal elastic lamina; in the slight development of the elastic tissue in the circular musculature of the media; and in the striking decrease in the longitudinal elastic fibers of the adventitia. He believed that as the artery decreased in size the elastica interna decreased in thickness until it finally disappeared entirely in the precapillary stage, leaving a vessel composed simply of a few strands of muscle fibers lined by a layer of endothelial cells.

Schäfer's<sup>2</sup> description in 1912 agreed with that of Triepel.

Binswanger and Schaxel<sup>3</sup> in 1917 studied the cerebral arteries in brains of individuals from birth to 60 years of age and concluded that at birth the structure of all arteries is complete and the later changes are those of quantity alone. As age advances the elements vary in their growth. By middle life the elastic tissue of the media and the elastica interna has stopped increasing. The muscle of the media continues to grow up to the 60th year. The collagenous tissue continues to increase and retains the greatest growing power. In older people, therefore, the cerebral vessels would show considerable increase in connective tissue at the expense of the rest of the wall elements. As the vessels decrease in size there is a decrease in the

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quantity and quality of the elements in the walls. The elastic fibers in the media disappear first, then the muscle fibers decrease, only the collagenous tissue remaining to surround the arteriole to the precapillary stage. This is in disagreement with Triepel who believed that muscle fibers could be detected around precapillaries.

Spielmeyer <sup>4</sup> in 1922 and Jacob <sup>5</sup> in 1927 agreed in their description of the cerebral vessels with Binswanger and Schaxel. However, they differed in their description of the capillaries which they believed contained an intima, elastica interna and a few adventitial fibers. Jacob described the cerebral arterioles as containing definite oblique muscle cells within their media.

Hackel <sup>6</sup> in 1928 studied the cerebral arteries in 13 cases varying in age from 15 days to 50 years, and described a structure quite similar to that described by Triepel. Hackel emphasized the relative scarcity of elastic fibers in the media as compared to the adventitia.

In 1931 Tuthill <sup>7</sup> made a study of the elastic layer of the cerebral arteries in 2 stillborn infants and in 24 children varying in age from birth to 14 years. He believed the size of the vessel determined the presence and the thickness of the elastica interna at birth. In the smaller arteries the elastic layer increases in thickness chiefly during the first 5 years, although it continues to grow throughout childhood, becoming quite a prominent part of the vessel wall.

With these earlier investigations in mind the present studies were undertaken in order to determine the normal histology of the cerebral vessels, as well as the normal variations in structure that may be encountered in the routine observation of normal brains in any single age group. For this investigation 70 apparently normal brains were selected, from our coroner's service, from individuals all of whom died of some extracerebral condition such as gunshot wound of the abdomen, crushed chest, and so on. The age groups of these individuals were as follows: 6 cases up to 10 years of age; 21 cases from 11 to 30 years of age; 20 cases from 31 to 50 years of age; 16 cases from 51 to 60 years of age; and 7 cases over 60 years of age.

During the early part of this investigation many blocks of tissue were taken from the various regions of the brain. It was necessary, therefore, to study a great many slides in each case. It was soon observed that the structure of the small cerebral arteries was quite constant in the various blocks studied, and that adequate observations could be made on fewer sections. Therefore, single blocks were

taken from representative areas throughout the cerebral cortex and the white substance and fixed in formalin. Sections were stained with the Weigert-Van Gieson stain, by the Mallory-Heidenhain technique (azocarmine), with Weigert's elastic tissue stain, and with the hematoxylin-eosin stain. For the study of muscle and fibrous tissue in the cerebral vessels staining by the Mallory-Heidenhain technique is invaluable since it demonstrates even the smallest connective tissue and muscle fibers. However, it does not differentiate between elastic and collagenous fibers. For this purpose Weigert's elastic tissue stain serves very well. Hematoxylin-eosin and Van Gieson stains were used to study the cellular structures.

The present study was limited to arteries varying in size from 20 to 150  $\mu$  in diameter. A detailed description of the larger vessels will be given, followed by a discussion of the various modifications that occur in the wall elements as the vessel decreases in size.

### NORMAL CEREBRAL ARTERIES

The small cerebral arteries are characterized by the thinness of their walls. In arteries measuring 150  $\mu$  in diameter the vessel wall is frequently less than 15  $\mu$  in thickness. The walls of these vessels may be divided roughly into the three coats ordinarily described for arteries, namely, interna, media and externa, although microscopic study of these vessels does not always reveal clear-cut divisions between these layers. The middle layer shows the greatest departure from the structure usually observed in the average small artery. It will, therefore, be described in some detail in a later paragraph.

*Intima:* The inner coat or intima is composed of an endothelial lining and an elastica interna. The latter is a thick, compact laminated structure and exhibits only a few fairly regular waves that project into the vessel lumen. This internal elastic lamina is both relatively and absolutely thicker in the cerebral vessels than in similar vessels elsewhere in the body. This relative thickness of the elastica interna tends to persist even into the smallest arteries. In many of these smaller vessels, although this structure retains its relatively large proportions, it frequently reveals certain changes in its tinctorial properties. Irregular segments of the elastica may fail to stain. In the smallest of vessels the elastic membrane decreases rapidly in thickness until it finally consists of a few strands of fibers that appear as granules in cross section.

*Media:* The media of most small extracerebral arteries is usually thick relative to the size of its lumen and consists mostly of circularly arranged flat bundles of smooth muscle. Numerous elastic fibers appear between the muscle cells forming a wide meshed network. Delicate collagenous strands, although present within the media, are few in number. They tend to follow the ramifications of the elastic fibers into the adventitia. The basic tissue within the media of the average small artery of a young individual consists, therefore, of smooth muscle, and the collagenous fibers are usually few and inconspicuous. The latter tissue does increase with advancing age, but only in vessels of very old individuals does it become a dominant element in the media. The middle layer of the small cerebral arteries presents quite a different picture. It is composed of a foundation of radially arranged, fine and coarse collagenous fibers which seem to fuse with one another. The collagenous tissue comprises a surprisingly large proportion of this lamina and in many cases makes up the greatest bulk of the wall (Fig. 1). Throughout this connective tissue there are found varying amounts of obliquely arranged muscle cells and nuclei. The muscle tissue is usually quite irregular in occurrence and is often entirely absent from a part or from the entire vessel wall. The elastic elements in the media are scarce.

There is a striking change in the middle layer as the vessel decreases in size. The structure of the wall becomes more simple. The elastic and muscle tissue rapidly disappear and are often difficult to find in vessels of  $70\mu$  in diameter. Often only an occasional muscle cell nucleus can be detected within an almost solid collagenous wall. Thus in the smaller vessels the entire media, regardless of the age of the patient, appears to be composed of collagenous connective tissue, which merges imperceptibly with the adventitia. At this stage, with the azocarmine stain, this lamina is a solid blue staining layer of tissue.

The media of the small cerebral arteries, therefore, possesses certain features that are quite different from those of similar sized vessels elsewhere in the body, namely, a relative paucity of both elastic and muscle tissue and a predominance of collagenous fibers. In all the smaller vessels, as well as in many of the larger ones of apparently normal young adults, the media is composed almost strictly of collagenous tissue. So striking is the appearance of this

layer, especially when stained by the Mallory-Heidenhain technique, that at first it was believed to be of pathological significance. Its uniform occurrence in the smaller arteries demonstrates that what appears to be fibrosis of the media is normal for cerebral arteries.

*Adventitia:* The adventitial layer of the small cerebral arteries is quite variable in size. In some cases it is composed only of a few strands of tissue, while in others it is equal in thickness to the adjoining media. As a rule it is quite thin and made up of a loose network of longitudinally and circularly arranged collagenous fibers. This lamina is well supplied with elastic fibrils. In the smaller arteries this outer layer of collagen becomes less and less distinct as an individual structure. It merges with the adjacent fibrous media to form a single layer.

### AGE CHANGES

*Intima:* With the advance of age numerous alterations begin to occur in all layers of the cerebral arteries. One of the first structures to show changes is the internal elastic lamina. Whereas this structure is usually a solid uniform band, it begins to show areas of reduplication as early as the latter part of the third decade. At first this consists of an irregular fraying of the solid, thick elastic lamina, forming tiny fine fibrils which project irregularly from various portions of the main lamina. This process results in a pseudoreduplication of the internal elastic lamina. True multiplication of the elastica interna does occur, but usually later in life — during the fourth decade and most frequently during the fifth and sixth decade (Fig. 2). This reduplication, however, is not the only change that occurs within this structure with the advance of age. Changes of equal frequency occur in the tinctorial properties of this membrane. It loses its ability to stain normally with the usual dyes and appears patchy and indistinct (Fig. 2).

Occasionally certain segments of the elastic lamina appear swollen. This swelling usually extends in an outward direction encroaching on the media. It is in these swollen areas that the first changes in the staining properties of this elastic tissue occur.

In advanced age large portions of the elastic membrane lose their normal staining properties. The thickened pale staining membranes, instead of appearing as uniform structures, are split not only into longitudinal structures but often also into transverse frag-

ments. This, no doubt, is a stage in the final disintegration of this membrane.

*Media:* The earliest change that is noticeable in the media of the cerebral arteries is an alteration quite similar to that which occurs as the vessel decreases in size, namely, a complete reduction in the quantity of its elastic and muscular elements. This change is quite conspicuous and is fairly complete early in life. When fibrosis of the media is complete it is usually impossible to differentiate this lamina from the adventitia, these two layer elements merging with one another to form a single structure. The cerebral vessels usually reveal a complete fibrosis of their media long before any preponderance of collagen begins to be apparent in this layer of the extracerebral vessels. Hence, such vessels in the younger age group must not be considered as a pathological finding but as a normal age process that occurs fairly early in life.

Other age changes occur within this layer. During the fifth and sixth decades many of the vessels show an indistinct staining of the medial elements. The heavy collagenous fibers lose their outline and become hyalinized. Similar changes occur in the adventitia which at this stage usually cannot be separated from the media (Fig. 3).

The extreme fibrosis and hyalinization of the vessel elements often fray the arterial wall and weaken it to such an extent that erythrocytes break through and escape from the lumen through the frayed elastica and vessel wall to the perivascular spaces, forming a ring of red cells around the vessel. It is not uncommon to find red cells scattered among the fragmented elements of a hyalinized vessel wall.

A final and more uncommon alteration that is observed in the media with advance of age is calcification of the wall elements. This change was rarely observed before the fifth decade. The calcium is at first deposited as irregular tiny particles in the outer portion of the media and inner adventitial layers (Fig. 4). The deposition may increase and become so complete that the entire wall is replaced by the calcium particles, which form a solid ring around the uninvolved lumen. This calcification is independent of any other change within the wall and may occur with or without hyalinization or fibrosis of the wall elements.

*Adventitia:* The age changes in the adventitia of the small cerebral arteries resemble closely those already described in the media. The

hyalinization that involves the media spreads also to the adventitia (Fig. 3). Calcification, when it occurs, spreads from within outward, at first involving the portion adjacent to the media but eventually replacing the entire wall (Fig. 4).

In the smallest arteries no differentiation can be made between the middle and outer vessel lamina, since the media early undergoes complete fibrosis and hence merges imperceptibly with the outer lamina.

*Basal Vessels:* As stated in a previous paragraph, when the small cerebral vessels alone are considered there is very little difference in their structure regardless of the part of the cerebrum considered. There is one possible exception to this statement and that concerns the vessels in the basal nuclei. As a rule these small arteries are thicker walled and contain somewhat more muscle tissue within their medial walls. The small arteries of  $50\ \mu$  have already lost enough muscle to make them indistinguishable from similar sized vessels elsewhere in the brain.

The age changes already described occur also in the basal vessels, only much more extensively and at an earlier age. Reduplication of the elastica interna can be seen as early as the third decade. The medial musculature disappears very rapidly so that these vessels rapidly assume a fibrous appearance. Hyalinization and calcification of the vessel wall are quite frequent and usually well advanced by the fourth decade. The calcification occurs in the same manner as described for the other cerebral arteries, only it occurs more rapidly and often completely replaces the vessel wall in individuals about 40 years of age.

## DISCUSSION

The changes occurring in the cerebral arteries with age have been mentioned in the literature, although in many cases their significance was overlooked. Tuthill,<sup>7</sup> Hackel,<sup>6</sup> and Triepel<sup>1</sup> all described the splitting of the elastica interna with age. Triepel gave a fairly complete description of the elastic reduplication but did not connect this alteration with the age of the patient. Tuthill believed that the abnormalities in the elastica interna were due to the effects of intoxication or infection. Hackel studied the cerebral arteries in 13 cases where the age varied from 15 days to 50 years. He described the splitting of the elastica interna as an old age phenomena.



Rosenblath<sup>8</sup> in studying a series of cases noticed that frequently the small cerebral arteries showed a fibrous tissue increase in their walls. In some vessels this fibrosis was quite extreme and caused the author to suspect that this change might be the cause of various nervous tissue alterations. He believed the fibrosis was caused by some type of chronic inflammation. Binswanger and Schaxel<sup>3</sup> also noticed the fibrous tissue increase in the cerebral arteries of older people. However, they carried their study farther and concluded that the various tissues in the arterial wall possess different growing powers. The collagen retains the greatest growing power and continues to increase after the other elements have ceased. Therefore, in older people the cerebral vessels would show a considerable increase in connective tissue, and a relative absence of elastic and muscle tissue.

It appears from our studies on normal brain tissue that the so-called medial fibrosis is the normal structure for the small cerebral arteries and that this fibrosis merely becomes more extensive with the advance of age. Elastic reduplication, hyalinization and calcification, on the other hand, occur only with advancing age and are not observed in the arteries of young individuals.

### CONCLUSIONS

1. The average small cerebral artery differs in structure from similar sized vessels elsewhere in the body in that it contains within its media a relative paucity of both elastic and muscle tissue and a predominance of collagenous fibers.

2. The very small cerebral arteries are composed almost strictly of collagenous tissue and may appear as a cerebral fibrosis.

3. With the advance of age the elastica interna of the cerebral arteries becomes reduplicated and frequently loses its normal tinctorial properties. The media undergoes a rapid fibrosis. It frequently shows a hyalinization and more rarely a calcification of all of its elements.

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## DESCRIPTION OF PLATE

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### PLATE 80

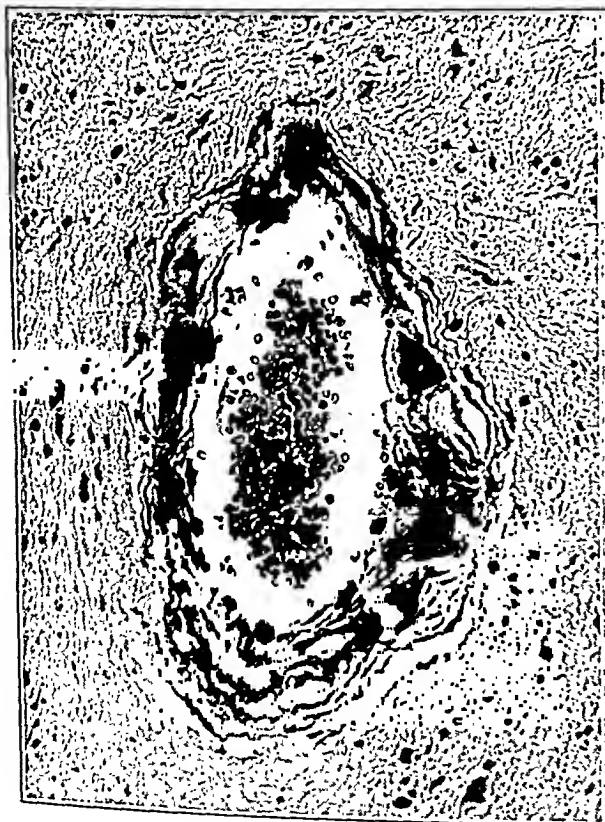
- FIG. 1. Structure of a normal cerebral blood vessel from an individual in the third decade of life. The entire wall is composed of collagenous connective tissue. Only a few cell nuclei are present within the wall elements. Hematoxylin and eosin stain.  $\times 1000$ .
- FIG. 2. Age changes within the elastica interna of a small cerebral artery. Note the reduplication and the patchy and indistinct staining of this membrane. Weigert's elastic tissue stain.  $\times 1000$ .
- FIG. 3. Hyalinization of a cerebral artery. The hyalin is deposited irregularly throughout the vessel wall. Hyalinization is quite extensive in this case and has replaced most of the wall elements. Hematoxylin-eosin stain.  $\times 1000$ .
- FIG. 4. Calcification of a cerebral artery. Note that the deposition of the calcium is limited to the outer portion of the media and the inner layer of the adventitia. The calcium has already formed an almost complete ring within the vessel wall. Hematoxylin-eosin stain.  $\times 1000$ .



1



2



3



4

Baker

Structure of Small Cerebral Arteries



# THE HISTOPATHOLOGY OF IDIOPATHIC THROMBOCYTOPENIC PURPURA HEMORRHAGICA \*

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## INTRODUCTION

Although the literature dealing with the clinical aspects of idiopathic thrombocytopenic purpura hemorrhagica has been voluminous, comparatively little attention has been given to its microscopic pathology. Histological reports of spleens removed surgically have been brief, inconclusive, and have shown a lack of uniformity in the findings. This has led to widely divergent theories often based on insufficient material. Adequate bone marrow studies have been curiously lacking and only a few reports of isolated cases, with examination usually of but one marrow, are to be found. Examination of other organs in autopsied cases has shown no common, significant pathological changes except the fundamental one of widespread hemorrhage.

The finding of strikingly similar histological changes in 2 cases of idiopathic thrombocytopenic purpura hemorrhagica, autopsied by us within a brief period of time, has led to a review of the literature and an investigation of the histopathology of this disease in such material as could be collected.

## REVIEW OF LITERATURE

Kaznelson<sup>1</sup> in two spleens removed surgically from cases of idiopathic thrombocytopenic purpura hemorrhagica found numerous megakaryocytes in the "pulp strands" whose presence, he believed, was due to embolic lodgment. The splenic follicles were small and germinal centers absent. Moderate numbers of neutrophilic and eosinophilic polymorphonuclear leukocytes were present in the sinuses. Occasional single platelets were seen in the pulp strands in only 1 case. Unfortunately he was unable to study the bone marrows in these cases.

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Minot<sup>2</sup> examined the bone marrow from a male who died of rapidly fulminating purpura of only 6 weeks duration. The megakaryocytes were plentiful and perhaps increased over normal numbers. He stated that he was unable to tell whether they had any definitely altered histological appearance.

Herzog and Roscher<sup>3</sup> reported 3 cases of secondary thrombocytopenic purpura hemorrhagica. In the 1st case the purpuric manifestations were secondary to massive bone marrow metastases from a primary liver cell carcinoma. The few megakaryocytes seen were apparently normal. The 2nd case (typhoid fever) showed active germinal centers in the splenic corpuscles and rare phagocytosis of platelets in the pulp. The marrow showed suppression of the neutrophilic elements and a lymphoid metaplasia. Megakaryocytes were rare and were thought to show nuclear changes. This case, they believed, showed functional bone marrow damage. The 3rd case, a syphilitic, showed an aplastic marrow, which was qualitatively normal but quantitatively deficient, and megakaryocytes were rare. The spleen had small follicles and showed occasional multinucleated cells in the pulp.

Brill and Rosenthal<sup>4,5</sup> reported 2 cases of idiopathic thrombocytopenic purpura hemorrhagica. One of the spleens was much enlarged, weighing 1400 gm., and was reported to show hyperplasia of the malpighian bodies and myeloid metaplasia. The second was moderately enlarged and showed only "hypertrophy" microscopically.

Jedlička and Altschuller<sup>6</sup> reviewed 2 cases. Following splenectomy 1 of these cases showed improvement, but in the other case death followed operation. The spleen in the latter case showed, microscopically, inactive germinal centers, many eosinophiles and a few isolated platelets. The bone marrow showed a predominance of neutrophilic myelocytes with very few myeloblasts. There were also many eosinophilic leukocytes and myelocytes. Megakaryocytes were numerous and the majority were reported to show dense structureless nuclei and a ragged, often vacuolated cytoplasm. Granules were irregularly scattered throughout the cytoplasm or gathered into groups. Sternal marrow biopsy of the 1st case showed numerous megakaryocytes with similar, but less marked changes. The spleen apparently was not examined.

Seeliger<sup>7</sup> examined the bone marrow from 2 cases of severe pur-

pura. In each case he studied and compared 200 megakaryocytes, which were present in increased numbers. A great majority contained no azure platelet-forming granules. The cells were poorly defined and vacuolated, and frequently showed degeneration of the nuclei, which contained many nucleoli. The cytoplasm was hyaline and filamented, and contained no cell inclusions. He stated that the findings in the spleen were not characteristic. Megakaryocytes were never found and increased numbers of platelets were seen in only one case.

An autopsied case was presented by Schmincke.<sup>8</sup> The spleen contained megakaryocytes, myelocytes and myeloblasts. Platelets were reported but not in increased numbers. The malpighian corpuscles were small. The femoral marrow showed normal granulopoiesis and erythropoiesis. Megakaryocytes were increased. Most of them were said to show structureless nuclei and often shadow forms. Their cytoplasm was granule-free, irregular and contained many lymphocytes and leukocytes which the author believed to be evidence of phagocytosis. He was tempted to think that megakaryocytes were present in the spleen as compensation for their destruction in the bone marrow, but concluded it was merely myeloid metaplasia.

Two cases were reported by Gaspar.<sup>9</sup> The marrow of 1 case showed increased numbers of megakaryocytes, most of which were interpreted as degenerated forms. There was a smaller proportion of normal appearing young megakaryocytes which, however, were not forming platelets. Only a few platelets were found in the spleen and none of them was phagocytosed. The femoral marrow of the 2nd case showed very few megakaryocytes, all of which were degenerated forms. The spleen contained no platelets.

In a series of collected spleens from cases of essential thrombocytopenic purpura hemorrhagica Gordon<sup>10</sup> found a uniform pathological picture. The follicles were unaffected and the pulp was said to be crowded with proliferating endothelial cells derived from the sinus endothelium. In addition he found phagocytic giant cells which he believed had not been reported previously.

Gerlach<sup>11</sup> reported an autopsied case of Werlhof's disease. The lymph follicles of the spleen were small. He could find no change in the sinus endothelium and found no myeloblasts, myelocytes or eosinophiles. Platelets and megakaryocytes were said to be few. Examination of the liver and lung revealed no megakaryocytes or



platelets. Myeloblasts and neutrophilic myelocytes formed the majority of cells in the femoral marrow. Adult leukocytes of all types were reported absent and there were very few nucleated red blood cells or erythroblasts. There were great numbers of megakaryocytes with large condensed nuclei. They possessed very little cytoplasm, no granules and, to the author, appeared degenerated. Only a few megakaryocytes possessed pseudopods and contained azurophilic granules. No mention was made as to the presence of platelets in the marrow but it was concluded that Wright's<sup>12</sup> theory of platelet formation was correct.

Merklen and Leriche<sup>13</sup> examined a spleen in which the findings are of questionable value because a diagnosis of aleukemic myelosis could not be ruled out.

Twenty-one clinical cases of hemorrhagic thrombocytopenia, with examination of the spleen in 7 cases and three autopsies, were reviewed by McLean, Kreidel and Caffey.<sup>14</sup> The spleens showed no significant changes. Moderate congestion of the sinuses was present in 2 cases and hyperplasia of the malpighian bodies in 1 case. The number of platelets varied from few to large platelet masses. The 3 autopsied cases showed no informative extrasplenic pathological changes except for cerebral hemorrhage in 2 of the cases.

Pemberton,<sup>15</sup> reporting a series of 57 splenectomies for essential thrombocytopenic purpura hemorrhagica, found, on microscopic examination of the spleen, no constant or distinctive pathological changes other than chronic congestion or chronic splenitis. Tuberculosis was present in 2 cases.

Lawrence and Knutti<sup>16</sup> examined the bone marrow in 6 cases of idiopathic thrombocytopenic purpura hemorrhagica. Four cases were clinically of the chronic type. The marrows were normal in 2 of these and megakaryocytes were said to be decreased in the others. The remaining 2 cases, clinically acute, showed hyperplastic marrows with morphological variations in the megakaryocytes.

Sternal biopsies of 4 cases of idiopathic thrombocytopenic purpura hemorrhagica and 1 case of purpura secondary to malignant diphtheria were examined by Willi.<sup>17</sup> In the former cases the megakaryocytes were not pathological in appearance but no platelets were seen. The case of malignant diphtheria showed vacuolization of the cytoplasm of the promegakaryocytes in the marrow. Again no platelets were found.

Krjukof<sup>18</sup> presented a case of Werlhof's disease with spontaneous recovery. Marrow biopsy showed numerous normal appearing megakaryocytes surrounded by many platelets.

Smith<sup>19</sup> reported 1 case successfully treated by splenectomy. Microscopically the spleen showed dilated sinuses with thickened walls. The malpighian bodies were of normal size and the germinal centers often contained degenerated macrophages.

Finally Levrat,<sup>20</sup> who has been the only author to attempt to establish a common pathological picture in spleens from cases of idiopathic thrombocytopenic purpura hemorrhagica, has reported the examination of a spleen from a case of his own and reviewed the descriptions of eleven other spleens reported in the French literature. His case and 5 others showed a hyperplasia of the splenic corpuscles with clear germinal centers in which were many mitoses. Many islands of myeloid tissue were found in the pulp and there was proliferation of the sinus endothelium. He believed these findings to be too common an occurrence to be mere coincidence but could not decide whether they were fundamental to the pathology of purpura or a secondary reaction to consecutive and repeated hemorrhages. The other spleens showed no common findings of significance. One of them contained large macrophages with cytoplasmic granules. This individual later came to autopsy and partial hematopoietic transformation of the lymph nodes was reported. The bone marrow was hyperactive and the megakaryocytes showed morphological alterations which were considered pathological. The spleen from his case showed scattered megakaryocytes which he apparently considered a part of the myeloid metaplasia.

### PATHOGENESIS

Frank<sup>21</sup> believed the disease to be due primarily to a marked platelet reduction through inhibition or destruction, with morphological changes, of the megakaryocytes by some noxa probably arising in the spleen. This theory has the most adherents.<sup>5-9</sup> Minot,<sup>2</sup> agreeing in part with this theory, suggested a specificity of the deleterious agent enabling it to act on the circulating platelets, on the megakaryocytes, or on the marrow as a whole. He stressed the similarity of certain cases of aplastic anemia to those of idiopathic thrombocytopenic purpura hemorrhagica. Kaznelson,<sup>1</sup> however,

believed that the reduction of blood platelets was brought about by an increased thrombolytic activity of the spleen. The increased number of megakaryocytes in the marrow, reported by other authors, he believed was a compensatory hyperplasia. On this hypothesis he introduced splenectomy as a therapeutic measure. In an attempt to explain the purpuric manifestations in cases without thrombocytopenia Glanzmann<sup>22</sup> was the first to postulate a hereditary qualitative deficiency of the platelets.

More recently, with accumulated evidence that thrombocytopenia is not the sole factor in the causation of hemorrhages, the theory has been evolved that in all cases a hyperpermeability of the capillary walls must exist. Brill and Rosenthal<sup>4, 5</sup> concluded that the disease was associated with a disturbed function of the capillary walls, in addition to a disordered state of the blood platelets. They suggested that the changes in the capillary wall might be due to a diminished number of platelets. Payne and Whitehead<sup>23</sup> later came to essentially the same conclusions. It was suggested by Salzman<sup>24</sup> that the spleen may destroy normal numbers of abnormally formed platelets. Krjukof<sup>18</sup> suggested that normally formed platelets may be retained within the capillaries of the bone marrow by a spasm of their walls. He also considered the possibility of some cases falling into the group of allergic manifestations. Combinations of these theories have been suggested by other authors.<sup>16, 25, 26</sup>

### MATERIAL AND METHODS

Our material consisted of the following autopsied cases: 5 cases of idiopathic thrombocytopenic purpura hemorrhagica; 3 cases of secondary thrombocytopenic purpura hemorrhagica; 14 cases of symptomatic purpura hemorrhagica; 2 cases of unclassified purpura hemorrhagica; 2 cases of aplastic anemia; 1 case of scurvy; 1 case of hemophilia; 1 case of hemorrhagic disease of the newborn; and 9 normal control bone marrows from cases of sudden death in the various age groups. Also included were spleens removed surgically from 6 cases of idiopathic thrombocytopenic purpura hemorrhagica; 2 cases of unclassified purpura hemorrhagica; and nine spleens from various conditions such as pernicious anemia, hemolytic jaundice, Banti's disease, cirrhosis of the liver, and so on. Of this material, 2 of the cases of idiopathic thrombocytopenic purpura

hemorrhagica, and 1 case of secondary thrombocytopenic purpura hemorrhagica were autopsied by us. The majority of the other material was taken from the autopsy and surgical cases of this laboratory. The remainder was collected from the Peter Bent Brigham Hospital, the Faulkner Hospital, Children's Hospital and from private cases of Dr. A. R. Kimpton.

Tissues from material in this laboratory were fixed in Zenker's fluid, cut at  $6\ \mu$  and stained with phloxine-methylene blue. The tissues from other sources were fixed in Zenker's fluid, stained with hematoxylin and eosin, and occasionally by Giemsa's method.

Bone marrows were studied by means of differential cell counts after the technique of Krumbhaar and Custer.<sup>27</sup> The optical system employed was a  $15\times$  ocular micrometer of the two parallel line variety, and a 2 mm. oil immersion objective.

Identification and tabulation of 500 cells was done by each author on every marrow and a mean taken. Very little discrepancy was noted between the individual counts. In order to avoid any personal factor a mechanical stage was used and the fields were chosen by moving the stage one division. However, fields containing large fat vacuoles, capillaries and bone trabeculae were avoided, when encountered, by moving the stage one division farther. The number of cells per field varied between 30 and 90 and averaged 60.

Because of the limited number of fields observed in this differential count an accurate index of the number of megakaryocytes present could not be determined, so megakaryocyte counts were made. Following the technique of Krumbhaar and Custer megakaryocytes were counted in 200 consecutive fields, thus giving their percentage in 12,000 cells. Comparison of the individual counts, however, revealed a wide discrepancy. Therefore, megakaryocytes were counted in 400 consecutive fields by each author, a mean taken, and the result expressed as percentage per 24,000 cells. By this technique there was no significant variation.

The megakaryocytes were divided into three types: young, adult and degenerated forms. Those interpreted as young forms were fairly large cells with single, large oval nuclei, occasionally indented and possessing a characteristically arranged, heavy chromatin network. The cytoplasm was scanty, pinkish gray and finely granular. Although pseudopods were not seen the cell outline was occasionally irregular. Adult megakaryocytes possessed a large multilobulated

nucleus with a chromatin network similar to that of the young forms. The cytoplasm was abundant, finely granular and generally projected several pseudopods. In the degenerated form the nucleus was represented by a structureless, dark, compact irregular mass. The cytoplasm varied in amount and was generally free of granules. The cell outline was irregular. It is of interest to note that inclusions of leukocytes and mononuclear cells were found in the cytoplasm of only the late adult and degenerated forms.

Special megakaryocyte stains were done after the technique of Wright<sup>12</sup> and that of Kingsley.<sup>28</sup> However, since these stains require special fixation and comparatively fresh tissue, it was only in three marrows and one spleen that satisfactory results were obtained. Various modifications of the Romanowsky stain (Giemsa, azure II eosin, and Pappenheim's method) were tried. Tissues were fixed in formalin, formalin-Zenker, mercuric chloride and saline, and also in methyl alcohol. With these various techniques no satisfactory results were obtained.

### GROSS PATHOLOGY

Of the 5 cases of idiopathic thrombocytopenic purpura hemorrhagica which were autopsied (35-412 and 35-480; and PBBH 24-116, 25-45 and 35-107) the outstanding gross finding was hemorrhage. There were widespread petechiae and ecchymoses of the skin and mucous membranes in all cases. In 3 cases there was hemorrhage into the serous cavities. In 2 cases in which the head was examined intracranial hemorrhage was found. Other organs involved by hemorrhage were heart, lungs, gastro-intestinal tract pancreas, renal pelves and adrenals. All the spleens were moderately enlarged except one (PBBH 25-45) and all weighed between 95 and 311 gm. The only other constant gross finding of interest was prominence of the malpighian corpuscles. In the 2 cases autopsied by us all the marrows were red and active, except the tibial marrow in 1 case (35-480) which was fatty. Of the other cases it was noticed that the femoral marrow was active in only 1 case (PBBH 25-45).

The 3 autopsied cases of secondary thrombocytopenic purpura hemorrhagica showed similar, widespread hemorrhagic manifestations in 1 case (36-168) and only petechial hemorrhages and ec-

chymoses in the other cases. Splenomegaly was present in only 1 case (PBBH 25-78) and in no case was there any gross abnormality of the spleen. All marrows were active in 1 case (36-168), a child 1 year of age. The femoral marrow was active in another case (PBBH 25-78). The primary lesions in these 3 cases were suppurative otitis media, acute and chronic pancreatitis and pyelonephritis, and hypertensive heart disease.

The 2 autopsied cases of unclassified purpura hemorrhagica are so grouped because of some abnormality of symptomatology, or incompleteness, or discrepancy of laboratory data which makes an exact diagnosis impossible. Both cases presented widespread hemorrhage and the spleens weighed 150 and 979 gm. respectively. The femoral marrow was red and active in 1 case (09-136) but was not examined in the other.

The gross pathological lesions in the cases of scurvy, hemophilia and hemorrhagic disease of the newborn, of the 2 cases of aplastic anemia, and of the 14 cases of symptomatic purpura hemorrhagica, are not of significance in this paper.

All of the six spleens removed surgically from cases of idiopathic thrombocytopenic purpura hemorrhagica were enlarged. Their weight varied from 155 to 390 gm., averaging 227 gm. Grossly they showed prominent malpighian corpuscles but were otherwise negative. The two spleens removed because of an unclassified purpura hemorrhagica were both enlarged. One weighed 300 gm. and the weight of the other was not recorded. The nine spleens removed for other conditions and used for controls showed varying gross lesions which are not pertinent.

### HISTOLOGY OF BONE MARROW

The various marrows from the cases of idiopathic thrombocytopenic purpura hemorrhagica were all active and cellular with the exception of one tibial marrow (35-480) which was inactive. The marrows of the unclassified, symptomatic and secondary purpuras were at least normally active. Those of the control cases were considered as normal. The differential cell counts and megakaryocyte counts are given in Tables I to VI.

## DIFFERENTIAL MARROW COUNTS

Differential marrow counts could not be done on all available marrows because many were too poorly stained to permit accurate identification of the cells, or there were too few cells in the sections. They were done on the vertebral, femoral and sternal marrows of 2 cases of idiopathic thrombocytopenic purpura hemorrhagica (35-412 and 35-480) and on the rib marrow of 35-480. In 3 cases of symptomatic purpura hemorrhagica the vertebral marrows were counted, and in 1 case of secondary thrombocytopenic purpura hemorrhagica the vertebral and sternal marrows were counted. All vertebral marrows of the 9 control cases were counted. The results of these counts are given in Tables I, II and III.

From these tables it may be concluded that no significant difference exists in the proportion of the various cell elements of the marrows from cases of idiopathic thrombocytopenic purpura hemorrhagica, as compared with those from secondary thrombocytopenic and symptomatic purpura hemorrhagica and normal marrows. There is, however, in the marrows of the first group of cases a slight preponderance of the more mature forms of the erythrocytic series. This is undoubtedly a compensatory phenomenon induced by the prolonged severe hemorrhages. It is to be noted especially that there is no significant change in the percentage of eosinophilic granulocytes. There is very little variation between the percentage composition of the cellular elements of the various marrows of an individual case. Although there is a moderate degree of variation between the percentage composition of cells in the normal marrows of the various age groups they cannot be correlated to form any significant trend. It is interesting to note that in marrow 32-102, a case of severe infection, there was toxic suppression of the granulocyte series, which is brought out very well by the table.

## MEGAKARYOCYTE COUNTS

Megakaryocyte counts could not be done on all available marrows because of the same factors encountered in the differential cell counts. They were done on all marrows from cases of idiopathic thrombocytopenic purpura hemorrhagica except 1 (PBBH 25-45) which was considered too fatty. Marrows from 5 cases of symptomatic purpura hemorrhagica were suitable for counting, and two

TABLE I  
*Idiopathic Thrombocytopenic Purpura Hemorrhagica*  
*Differential Marrow Counts % Based on 500 Cells*

Case No.	Sex	Age	Bone marrow	Undifferentiated cells	Granulocyte series									Erythrocyte series				Thrombocyte series				Lymphocytes	Plasma cells	
					Premyelocytes	Eosinophilic premyelocytes	Myelocytes	Eosinophilic myelocytes	Metamyelocytes	Eosinophilic metamyelocytes	Segmented cells	Eosinophilic segmented cells	Total percentage	Stem cells	Erythroblasts	Nucleated reds	Total percentage	Young megakaryocytes	Adult megakaryocytes	Degenerated megakaryocytes	Total percentage			
35-412	F	23 yrs.	Vertebral	2.0	8.6	1.0	20.6	2.6	9.4	0.8	6.0	0.6	49.6	9.2	15.2	22.2	46.6	0.2	0.2	0.0	0.4	1.2	0.0	0.2
35-412	F	23	Femoral	2.0	8.2	0.4	19.2	1.0	14.8	1.2	6.2	0.4	51.4	3.4	13.4	25.4	42.2	0.0	0.8	0.0	0.8	3.2	0.0	0.4
35-412	F	23	Sternal	1.8	4.0	0.2	16.4	3.4	16.8	0.4	7.2	0.4	48.8	4.0	13.4	30.8	48.2	0.0	0.0	0.0	0.0	1.2	0.0	0.0
35-480	M	19	Vertebral	2.7	12.4	0.0	20.7	1.6	13.1	0.8	1.9	0.0	50.5	3.6	10.1	30.4	44.1	0.1	0.6	0.1	0.8	1.7	0.0	0.2
35-480	M	19	Femoral	2.8	4.8	0.6	14.8	1.2	10.0	1.0	2.4	0.4	35.2	4.8	12.2	43.2	60.2	0.4	0.0	0.2	0.6	0.6	0.0	0.6
35-480	M	19	Rib	4.2	10.2	0.2	16.4	0.6	15.2	0.6	1.4	0.2	44.8	9.2	10.6	27.2	47.0	0.0	0.0	0.6	0.6	3.2	0.0	0.2
35-480	M	19	Sternal	2.4	5.0	0.4	13.0	2.0	8.0	0.4	2.6	0.2	31.6	1.8	15.6	47.4	64.8	0.0	0.2	0.2	0.4	0.4	0.0	0.4



TABLE II

*Symptomatic and Secondary Purpura Hemorrhagica*  
*Differential Marrow Counts % Based on 500 Cells*

Case No.	Sex	Age	Bone marrow	Granulocyte series								Erythrocyte series				Thrombocyte series					Reticulo-endothelial cells	Lymphocytes	Plasma cells	Cause of death		
				Premyelocytes	Eosinophilic premyelocytes	Myelocytes	Eosinophilic myelocytes	Metamyelocytes	Eosinophilic metamyelocytes	Segmented cells	Eosinophilic segmented cells	Total percentage	Stem cells	Erythroblasts	Nucleated rebs	Total percentage	Young megakaryocytes	Adult megakaryocytes	Degenerated megakaryocytes	Total percentage						
32-102	F	40 yrs.	Vertebral	2.4	10.8	0.0	19.8	0.4	5.0	0.0	0.4	0.0	36.4	6.4	31.2	17.6	55.2	0.4	0.6	0.4	0.4	1.4	3.0	0.0	1.6	Pneumonia, purpura
28-209	F	65	Vertebral	4.4	8.8	0.0	26.2	0.2	10.2	0.4	6.2	0.0	52.0	7.0	20.8	13.2	41.0	0.0	0.2	0.0	0.0	0.2	1.8	0.0	0.6	Parotitis, purpura
26-35	F	40	Vertebral	5.0	13.8	0.0	21.8	1.4	17.8	1.2	3.6	0.0	59.6	2.4	19.8	8.2	30.4	0.0	0.6	0.0	0.0	0.6	3.4	0.0	1.0	Lobar pneumonia, purpura
36-168	M	1	Sternal	2.4	5.8	0.8	18.0	1.2	12.6	0.8	5.2	1.2	45.6	7.2	12.8	30.4	50.4	0.0	0.4	0.0	0.2	0.6	1.0	0.0	0.0	Otitis media, secondary thrombocytopenic purpura
36-168	M	1	Vertebral	2.4	8.8	0.4	22.0	2.0	11.6	0.8	5.4	0.4	51.4	7.6	18.8	17.6	44.0	0.0	0.4	0.0	0.2	0.6	1.6	0.0	0.0	Otitis media, secondary thrombocytopenic purpura

TABLE III

## Normal Marrow Controls

## Differential Marrow Counts % Based on 500 Cells

Case No.	Sex	Age	Bone marrow	Undifferentiated cells	Granulocyte series								Erythrocyte series				Thrombocyte series				Reticulo-endothelial cells	Lymphocytes	Plasma cells	Cause of death	
					Premyelocytes	Eosinophilic premyelocytes	Myelocytes	Eosinophilic myelocytes	Metamyelocytes	Eosinophilic metamyelocytes	Segmented cells	Eosinophilic segmented cells	Total percentage	Stem cells	Erythroblasts	Nucleated reds	Total percentage	Young megakaryocytes	Adult megakaryocytes	Degenerated megakaryocytes					Total percentage
34-602	F	34 yrs.	Vertebral	1.0	8.6	0.6	19.8	5.6	13.6	0.0	7.4	0.0	55.6	8.4	15.2	18.2	41.8	0.0	0.4	0.6	1.0	0.4	0.0	0.2	Pulmonary embolism (20 days post-partum)
35-577	F	24	Vertebral	1.8	8.4	0.8	15.4	4.4	14.2	1.4	8.6	0.4	53.6	10.0	16.0	17.6	43.6	0.2	0.0	0.2	0.4	0.6	0.0	0.0	No anatomical cause (postoperative)
35-481	F	9 mo.	Vertebral	2.4	17.2	1.0	11.8	0.6	10.4	1.4	6.6	1.0	50.0	9.4	21.6	15.2	46.2	0.4	0.2	0.0	0.6	0.8	0.0	0.0	No anatomical cause
35-110	M	33	Vertebral	2.2	9.6	0.6	18.8	1.6	6.0	0.8	1.6	1.0	40.0	7.6	17.0	31.8	56.4	0.2	0.2	0.2	0.6	0.8	0.0	0.0	Syphilitic aortitis, polycystic kidneys
35-284	M	2	Vertebral	1.2	10.0	0.2	21.8	0.0	6.0	0.4	1.2	0.0	39.6	13.4	19.8	24.4	57.6	0.0	0.0	0.4	0.4	0.6	0.0	0.6	Hydrocephalus, pulmonary infarcts
35-343	M	38	Vertebral	2.0	8.8	0.2	17.8	3.2	6.4	0.8	6.6	0.6	44.4	12.4	16.2	22.0	50.6	0.2	0.4	0.0	0.6	2.0	0.0	0.4	No anatomical cause
34-676	M	54	Vertebral	2.6	10.6	0.6	14.4	1.2	12.6	0.4	9.0	0.0	48.8	11.4	16.0	19.2	46.6	0.2	0.4	0.0	0.6	1.2	0.0	0.2	Pulmonary embolism (unhealed fractures of tibia and fibula)
34-467	M	11 mo.	Sternal	1.0	7.2	0.8	14.2	1.2	5.4	0.4	2.4	0.2	31.8	7.6	33.6	24.6	65.8	0.4	0.0	0.0	0.4	1.0	0.0	0.0	Foreign body in bronchus, atelectasis
34-146	F	18	Vertebral	0.8	10.2	0.2	16.4	2.0	7.6	1.8	0.6	0.8	39.6	9.8	14.2	34.6	58.6	0.0	0.2	0.2	0.4	0.6	0.0	0.0	No anatomical cause (postoperative)

marrows of 1 case of secondary thrombocytopenic purpura hemorrhagica were counted. Vertebral marrows on both cases of unclassified purpura were counted as were the vertebral marrows of the 9 normal controls. The results are given in Tables IV, V and VI.

TABLE IV  
*Megakaryocyte Count*  
*Idiopathic Thrombocytopenic Purpura Hemorrhagica*  
*Percentage per 24,000 Cells Seen in 400 Fields*

Case No.	Sex	Age	Bone marrow	Total count		Megakaryocytes		
						Young	Adult	Degenerated forms
		yrs.		No.	%	%	%	%
35-412	F	23	Vertebral	230	0.920	16	43	41
35-412	F	23	Sternal	109	0.436	21	41	38
35-412	F	23	Femoral	137	0.548	19	53	28
35-480	M	19	Vertebral	72	0.288	17	62	21
35-480	M	19	Sternal	17	0.068	0	12	88
35-480	M	19	Femoral	34	0.136	15	50	35
PBBH								
24-116	M	26	Vertebral	94	0.380	8	47	45
PBBH								
35-107	F	40	Rib	73	0.292	18	61	21

From a study of the megakaryocyte counts it is seen that the total percentage of megakaryocytes in the normal marrow controls is surprisingly uniform, varying only from 0.24 per cent to 0.423 per cent. In all cases the young forms comprise the smallest group. Adult and degenerated forms appear to alternate almost equally in preponderance.

In the marrows from the cases of idiopathic thrombocytopenic purpura hemorrhagica, however, marked variation is seen, both between different cases and between different marrows of the same case. The vertebral marrow in case 35-412 contains more than twice the number of megakaryocytes present in the highest of the normal controls. The sternal and femoral marrows of the same case show only a slight increase over normal. The count of the vertebral marrow in case 35-480 is in the low normal range, while the sternal and femoral marrows of the same case are definitely below normal. The marrows of the remaining 2 cases are within normal limits.

Both marrows from the case of secondary thrombocytopenic pur-

TABLE V  
Megakaryocyte Count  
Secondary Thrombocytopenic and Symptomatic Purpura  
Percentage per 24,000 Cells Seen in 400 Fields

Case No.	Sex	Age	Bone marrow	Total count		Megakaryocytes			Cause of death
				No.	%	Young	Adult	Degenerated forms	
26-35	F	40 yrs.	Vertebral	79	0.314	%	%	%	Lobar pneumonia, purpura
28-209	F	65	Vertebral	26	0.102	15	43	42	Parotitis, purpura
32-102	F	40	Vertebral	171	0.682	20	51	29	Pneumonia, purpura
36-168	M	1	Vertebral	146	0.582	17	66	17	Otitis media, secondary thrombocytopenic purpura
36-168	M	1	Sternal	158	0.630	20	50	30	Otitis media, secondary thrombocytopenic purpura
C 28-131	M	11 mo.	Rib	116	0.464	19	48	33	Septic hip, malnutrition, purpura
C 32-158	M	4 mo.	Vertebral	44	0.176	13	40	47	Streptococcus septicemia, otitis media, purpura
						7	59	34	

Unclassified Purpura

09-136	F	20	Vertebral	122	0.485	22	54	24	Multiple hemorrhages, bronchopneumonia
PBBH 27-123	M	49	Vertebral	56	0.224	16	29	18	Purpura hemorrhagica

TABLE VI  
Megakaryocyte Count  
Normal Marrow Controls  
Percentage per 24,000 Cells Seen in 400 Fields

Case No.	Sex	Age	Bone marrow	Total count		Megakaryocytes			Cause of death
				No.	%	Young	Adult	Degenerated forms	
34-602	F	34	Vertebral	93	0.370	3	22	75	Pulmonary embolism (20 days postpartum)
35-577	F	24	Vertebral	106	0.423	22	45	33	No anatomical cause (postoperative)
35-481	F	9 mo.	Vertebral	61	0.246	21	42	37	No anatomical cause
35-110	M	33	Vertebral	75	0.300	10	33	57	Syphilitic aortitis, polycystic kidneys
35-284	M	2	Vertebral	87	0.348	11	41	48	Hydrocephalus, pulmonary infarcts
35-343	M	38	Vetrebral	99	0.346	17	42	41	No anatomical cause
34-676	M	54	Vertebral	87	0.348	11	63	26	Pulmonary embolism, unhealed fractures of tibia and fibula
34-467	M	11 mo.	Sternal	62	0.254	14	48	38	Foreign body in bronchus, atelectasis
34-146	F	18	Vertebral	76	0.304	17	36	47	No anatomical cause (postoperative)

pura hemorrhagica show moderate elevation of the total megakaryocyte percentage. Of the cases of symptomatic purpura hemorrhagica 2 show moderately increased numbers, 1 a normal number, and 2 a definitely diminished number of megakaryocytes.

Of the 2 cases of unclassified purpura hemorrhagica 1 shows a slight increase and the other an equally slight decrease of the total megakaryocyte count.

Although it is dangerous to generalize from a limited number of cases, from study of the percentage of the three age groups of megakaryocytes in the four groups of purpura hemorrhagica it would seem that with two exceptions a definite trend from normal is evident. There appears to be a distinct increase of young forms, together with a slight increase in adult forms, and a corresponding diminution of the degenerated megakaryocytes.

With the stains employed (phloxine-methylene blue, hematoxylin-eosin and Giemsa) no morphological abnormality was noted in the megakaryocytes observed in these counts. In three marrows (femoral (35-480) and femoral and tibial (36-168)), we were able to stain megakaryocytes specifically by Wright's<sup>12</sup> technique. In both cases the cytoplasm of all the megakaryocytes except the degenerated forms contained abundant azurophilic granules. Many pseudopods were seen and platelets could be seen breaking off from them in normal fashion, after the illustrations of Wright. Many of the actively platelet-forming cells were so small as to make it questionable whether or not they could be identified with routine stains.

Many platelets were seen scattered throughout the marrow, and many were grouped near disintegrating pseudopods. The majority of the platelets were of normal size with, however, a few giant forms being noted.

#### HISTOLOGY OF THE SPLEEN

On histological examination of the spleens from the 11 cases of idiopathic thrombocytopenic purpura hemorrhagica, fairly constant pathological changes were found. The germinal centers in all cases but 2 were enlarged and active, or hyperactive. The germinal centers in 2 cases only showed areas of necrosis of lymphocytes, fragmentation and phagocytosis of nuclei, occasional infiltration of polymorphonuclear leukocytes and clumps of fibrin (the so-called toxic reaction). In all cases but 2 there was an increase, often marked, in

the numbers of neutrophilic or eosinophilic polymorphonuclear leukocytes, or both, in the sinuses of the pulp.

The most significant finding, in our opinion, was the almost constant presence of megakaryocytes in the sinuses of the pulp. Although in most instances they were comparatively rare, in one spleen they were encountered very frequently and in two others they were surprisingly numerous, as many as four being seen in one high power field. All the cells seen appeared to belong to the young or adult group. With the Kingsley<sup>28</sup> stain, in one spleen (S35-2768 — 35-480) we were able to demonstrate the specific granules in the cytoplasm of these cells. Although pseudopods could be demonstrated, no platelets were positively identified. Whether this was due to staining idiosyncrasy or whether they were actually absent, we are unable to say.

With this finding of megakaryocytes in the sinuses a careful search was made in all spleens for hematopoiesis. In 1 case frequent nucleated red blood cells and myelocytes with occasional mitotic figures were found, and in another case there were several eosinophilic myelocytes and metamyelocytes. However, no stem or early cells of either series were found. Furthermore, the constant location of the megakaryocytes free in the sinuses points to their presence as being an embolic manifestation.

Proliferation of the cells of the sinus endothelium, so frequently mentioned by other authors, was not encountered. When the sinuses are comparatively free of blood, however, the cells of the lining endothelium assume a cuboidal shape, become more prominent, and may simulate hyperplasia.

The above mentioned changes are summarized briefly in Table VII.

The sections of the spleen in 1 of the 4 cases of unclassified purpura hemorrhagica were too faded for accurate interpretation. The remaining 3 all showed enlarged, active germinal centers with toxic reaction present in 2 cases. All showed moderately increased numbers of neutrophilic polymorphonuclear leukocytes in the sinuses, and also an increase in eosinophiles. Rare megakaryocytes were seen in only one spleen. In another scattered areas of erythropoiesis and granulopoiesis were found.

One of the 3 cases of secondary thrombocytopenic purpura hemorrhagica (36-168) was that of severe sepsis. The spleen showed large,

TABLE VII

*Data on Spleens from Cases of Idiopathic Thrombocytopenic Purpura Hemorrhagica*

Case No.	Weight of spleen	Gross description	Microscopic examination						Other data
			Germinal centers	Toxic reaction	Polymorpho-nuclear leukocytes	Eosinophiles	Megakaryocytes	Hemato-poiesis	
S 33-1368	gm. 390	Prominent follicles	Occasional large active	None	Many	Many	Occasional	None	Groups of macrophages laden with fat
S 35-873	155	Negative	Occasional active	None	Moderate	Moderate	Rare	None	
S 35-2966	175	Prominent follicles	Large active	None	Moderate	Few	Rare	None	
F 35-367	220	Negative	Large active	None	Rare	Occasional	None	None	Congestion of sinuses
F 34-373	175	Negative	Large active	None	Moderate	Many	Frequent	None	
S 24-92	250	Prominent follicles	Hyperactive	Frequent	Many	Moderate	One	None	
S 35-2768 (35-480)	300	Prominent follicles	Large, often active	None	Many	Few	Many	None	Trabeculae slightly thickened
35-412	220	Prominent follicles	Large active	None	Moderate	Many	Many	None	Congestion of sinuses, edema
PBBH	200	Negative	Occasional large active	None	Many	Few	Occasional	None	Eosinophiles, myelocytes and metamyelocytes
S 24-1002	95	Negative	Inactive	None	Few	Rare	Rare	None	Frequent nucleated reds, many myelocytes with occasional mitotic figures
PBBH 25-45	311	Prominent follicles	Active	Present	Moderate	Few	Several	None	Many vacuolated macrophages
PBBH 35-933									



active germinal centers with a toxic reaction, greatly increased numbers of neutrophilic polymorphonuclear leukocytes in the sinuses of the pulp, and very rare eosinophilic leukocytes. The sinuses contained comparatively little blood, thus making the endothelial lining cells clearly visible and prominent. One megakaryocyte was seen and no hematopoiesis was present. Another case (PBBH 25-78) was that of purpura in a case of long standing hypertension. Here the germinal centers of the malpighian corpuscles of the spleen were inactive and the vessels were hyalinized. Relatively few neutrophilic and eosinophilic polymorphonuclear leukocytes were present, and no megakaryocytes or areas of hematopoiesis could be found. The 3rd case (31-135) in which the purpura was again secondary to infection showed no microscopic abnormalities of the spleen.

In the 14 autopsied cases of symptomatic purpura secondary to various pathological processes sections of spleen were available for examination in all but 1 case. In all cases where the underlying pathology was overwhelming sepsis, notably septicemia and pneumonia, there were greatly increased numbers of neutrophilic polymorphonuclear leukocytes in the sinuses of the pulp. In 3 of these cases occasional megakaryocytes were seen. In all 14 the malpighian corpuscles were small and the germinal centers inactive. In only 2 did they show a toxic reaction. In 1 case eosinophilic leukocytes were prominent. There were clumps of stem cells in one spleen and eosinophilic myelocytes and metamyelocytes in another. Otherwise there was no evidence of hematopoiesis. Another finding in 2 unrelated cases was the occurrence of macrophages filled with phagocytosed red blood cells in the sinuses of the pulp.

In order to evaluate further the microscopic findings in the spleens from cases of idiopathic purpura hemorrhagica nine spleens removed surgically as a therapeutic measure in various pathological conditions were used as controls. Five of these had enlarged malpighian corpuscles with active germinal centers, which in 2 of the cases showed a toxic reaction. Moderate numbers of neutrophilic polymorphonuclear leukocytes were present in 3 cases and were slightly increased in 2 others. In only 1 of these were there more eosinophilic leukocytes than normal. In 3 cases rare megakaryocytes were found. In 1 of these spleens the germinal centers were active and in another there was a slight increase of neutrophilic polymorpho-

nuclear leukocytes in the sinuses of the pulp. In the 3rd case there were a few infarcts. There were no other abnormalities. It is of interest that each of these spleens was removed from a young individual, 10, 25 or 27 years of age, respectively. Also, in each case there was an antecedent history of appendectomy, which in 2 cases was followed by a subphrenic abscess. At the time of splenectomy in each case there were dense adhesions surrounding the liver and spleen, and in 2 cases the liver was noted to be considerably reduced in size at operation. These 3 cases seem to form a clinical group. They all showed megakaryocytes in the splenic sinuses, the significance of which is not clear. Careful search revealed no evidence of hematopoiesis. In two spleens removed from patients with cirrhosis of the liver of another type no megakaryocytes were found.

In a case of Banti's disease there were many large, often multinucleated macrophages containing red blood cells in the sinuses. Moderate hemosiderosis was present in a case of pernicious anemia.

In the spleens from the 2 cases of aplastic anemia that came to autopsy one showed large, active malpighian corpuscles with an occasional toxic reaction. The sinuses contained very few neutrophilic polymorphonuclear leukocytes but numerous phagocytic macrophages. One megakaryocyte was seen. The other case showed occasional masses of organisms in the sinuses with rare leukocytes and no megakaryocytes. The follicles were inactive and the arteriolar walls showed extreme hyalinization.

The spleen from the case of hemophilia showed a toxic reaction of the germinal centers of the malpighian corpuscles. There were numerous plasma cells in the sinuses of the pulp but no evidence of hematopoiesis. Several scattered megakaryocytes were seen. This latter finding is in accord with that of Custer and Krumbhaar,<sup>29</sup> who found megakaryocytes in the splenic sinuses in 2 of their 3 cases of hemophilia.

No sections of spleen were available from the case of scurvy, and the spleen from the case of hemorrhagic disease of the newborn showed no pathological changes of interest.

#### HISTOLOGY OF OTHER ORGANS

The only finding of interest in other organs, apart from hemorrhage, was the occurrence of megakaryocytes in cases of idiopathic

thrombocytopenic purpura hemorrhagica. In 2 of the cases (35-412 and 35-480) they were present in large numbers in the sinuses of the liver and lymph nodes and in the capillaries of the lung. In a 3rd case (PBBH 35-107) the liver only was examined. Several megakaryocytes were seen in the sinuses. This occurrence of megakaryocytes in other organs in addition to the spleen, together with their location always free in blood spaces, excludes any explanation for their presence but that of embolic lodgment. The 2 other autopsied cases of idiopathic thrombocytopenic purpura hemorrhagica showed no megakaryocytes in the liver.

No megakaryocytes were seen in the livers of autopsied cases of unclassified and secondary thrombocytopenic purpura hemorrhagica, symptomatic purpura hemorrhagic, aplastic anemia, hemophilia or scurvy. No evidence of extramedullary hematopoiesis was found in any of the organs examined.

## DISCUSSION

From the bone marrow studies it may be inferred that, in certain cases at least, there is no essential difference in the cellular composition of the marrows from cases of idiopathic thrombocytopenic purpura hemorrhagica and other types of purpura hemorrhagica and also from presumably normal marrows.

The megakaryocytes, as seen by routine stains, appear morphologically normal. Specific stains have shown that in 1 case of idiopathic thrombocytopenic purpura hemorrhagica and 1 case of secondary thrombocytopenic purpura hemorrhagica they are apparently functionally normal. This finding is in marked contrast to those of Jedlička and Altschuller,<sup>6</sup> Seeliger,<sup>7</sup> Schmincke,<sup>8</sup> Gaspar,<sup>9</sup> Gerlach,<sup>11</sup> and Willi.<sup>17</sup> Although the latter found morphologically normal megakaryocytes in his 4 cases of idiopathic thrombocytopenic purpura hemorrhagica he stated definitely that there was a temporary complete arrest of platelet formation by the promegakaryocytes, which he considered to be the only platelet-forming type. In our own experience the platelets and megakaryocyte granules have been found to be extremely fragile and very susceptible to destruction by postmortem change and certain methods of fixation. This may possibly partially explain the discrepancy in the findings.

Megakaryocyte counts, compared to those of normal controls,

show increase, decrease or normal numbers. The one constant finding is a preponderance of young and adult forms. This suggests a functional hyperplasia of the megakaryocytes.

We have found a fairly uniform microscopic picture in the spleens of idiopathic thrombocytopenic purpura hemorrhagica. This consists of enlarged, active germinal centers with toxic reaction occurring only rarely. In the majority of the cases there are increased numbers of neutrophilic and eosinophilic polymorphonuclear leukocytes in the splenic sinuses. This increase of eosinophiles is of interest because of the not infrequent slight eosinophilia of the circulating blood and because of the normal numbers of eosinophilic granulocytes in the bone marrows of individuals with idiopathic thrombocytopenic purpura hemorrhagica. The most important, almost constant finding which seems to have been noted but rarely by other authors is the presence of megakaryocytes in the splenic sinuses. This point will be discussed at greater length below.

These findings of enlarged, active germinal centers, increased neutrophilic and eosinophilic polymorphonuclear leukocytes, and the presence of megakaryocytes are met with in other types of purpura and in other pathological conditions, but seldom are they all apparent in the same spleen. We feel that this picture is fairly constant and diagnostic.

Kaznelson<sup>1</sup> found megakaryocytes in two spleens and in the circulating blood of two patients. He interpreted their presence as being due to embolic lodgment and thought this reflected functional hyperplasia of the megakaryocytes in the bone marrow. Our finding of megakaryocytes in the splenic sinuses, in the sinuses of the liver and lymph nodes, and in the capillaries of the lung, in cases of idiopathic thrombocytopenic purpura hemorrhagica, together with the absence of any extramedullary hematopoiesis, would lead us to concur with Kaznelson.

Almost all of the extramedullary megakaryocytes seen were of the young or adult type. Their presence can be explained in only one of two ways. They may represent a maturational arrest of platelet formation, with a throwing out into the blood stream of the functionally active megakaryocytes, or there may be merely a functional hyperplasia of megakaryocytes in the bone marrow and a throwing off of young forms in an effort to compensate for an increased destruction of circulating platelets. This condition would

be analogous to that seen in the erythrocytes in hemolytic anemia. The finding of a slightly increased proportion of young megakaryocytes in the marrow and the demonstration of apparently normal platelet formation favors the latter explanation.

The significance of the finding of varying numbers of megakaryocytes in the marrow is not clear. A possible explanation is that the marrows in which fewer megakaryocytes are found may be showing an early exhaustion of the megakaryocyte series. In this respect it is of interest that the patient whose marrow showed the highest megakaryocyte count came into the hospital in an acute relapse and died in a few hours. The other cases had been hospitalized over a much longer period of time. This offers another explanation for the marrow findings of other authors, in that the megakaryocytes may have been in the end stages of exhaustion. If this be the mechanism the megakaryocyte counts should show a much higher proportion of the degenerated forms. Our counts, however, offer no support to this theory.

Although our findings lead us to believe that the cause of the thrombocytopenia in idiopathic thrombocytopenic purpura hemorrhagica is increased destruction of the platelets rather than inhibition of their formation, we have no evidence as to where this occurs. From clinical evidence, however, the spleen plays an important rôle.

The variation in size and appearance of platelets, so frequently described by other authors, does not necessarily imply that they are functionally deficient. It may also indicate a premature hurried advent into the circulation. Whether or not these platelets are qualitatively defective would seem to be a question for physiological rather than histological investigation.

The theory introduced by Minot<sup>2</sup> that toxins may at times act only on the circulating platelets is strongly supported by our case of secondary thrombocytopenic purpura (36-168). In this case there was a known infection (acute mastoiditis) and a severe thrombocytopenia, but the megakaryocytes in the marrow were increased and actively forming platelets. If this be the mechanism in many cases of purpura secondary to severe infection, the comparatively rare occurrence of megakaryocytes in the blood spaces of the viscera may be explained by the brief duration of this disease as contrasted to idiopathic thrombocytopenic purpura hemorrhagica.

From clinical and laboratory data it is obvious that thrombopenia

is not the sole factor in the causation of hemorrhages. Whether there is a concomitant qualitative defect of the platelets or a capillary hyperpermeability, or both, we are unable to say.

### CONCLUSIONS AND SUMMARY

Differential cell counts have shown no fundamental differences in the bone marrow from cases of idiopathic thrombocytopenic purpura hemorrhagica, various other forms of purpura hemorrhagica and normal controls. Megakaryocyte counts in all forms of purpura show no constant variation except a predominance of the functionally active forms. Under proper technical conditions active platelet formation was demonstrated in the bone marrow from 1 case of idiopathic and 1 case of secondary thrombocytopenic purpura hemorrhagica. A fairly uniform microscopic picture in the spleen and the presence of megakaryocytes in the various organs in cases of idiopathic thrombocytopenic purpura hemorrhagica is reported. The theories of pathogenesis are presented and evidence is introduced that thrombocytolysis is the cause of the thrombocytopenia. It is suggested that this in turn causes a compensatory hyperplasia of the megakaryocytes in the bone marrow. The literature on the histopathology of idiopathic thrombocytopenic purpura hemorrhagica is briefly reviewed.

NOTE: We acknowledge our indebtedness to Dr. S. B. Wolbach of the Department of Pathology, Harvard Medical School, Dr. J. B. Hazard of the Faulkner Hospital, and to Dr. A. R. Kimpton for the use of their material contained in this report; and also to Dr. F. Parker, Jr., for his constructive criticism and advice during the progress of this work.

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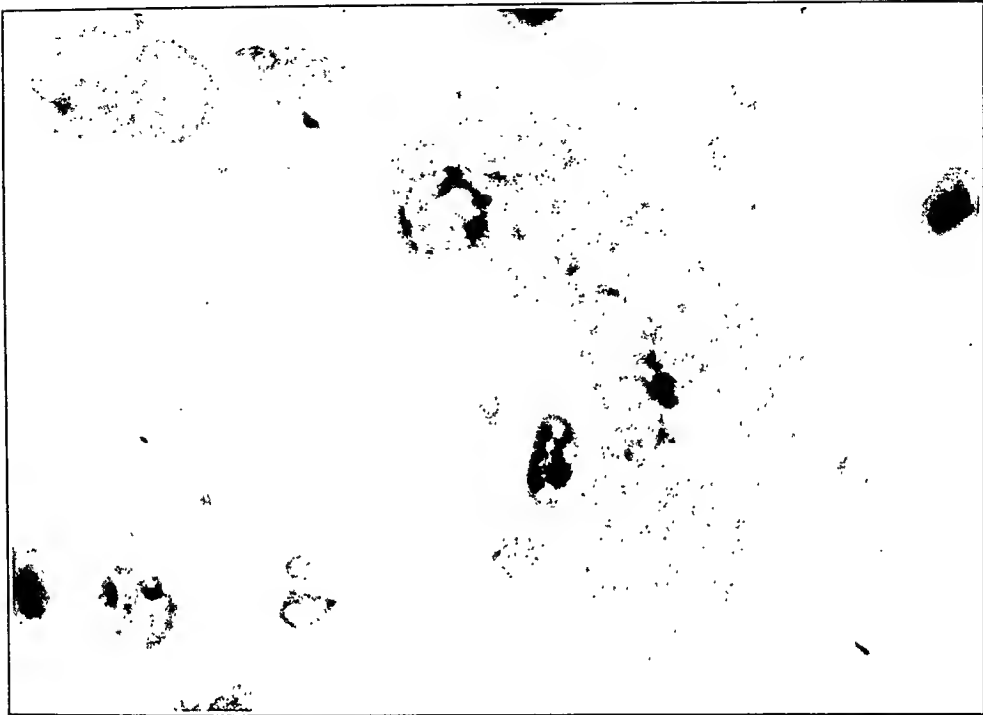


## DESCRIPTION OF PLATES

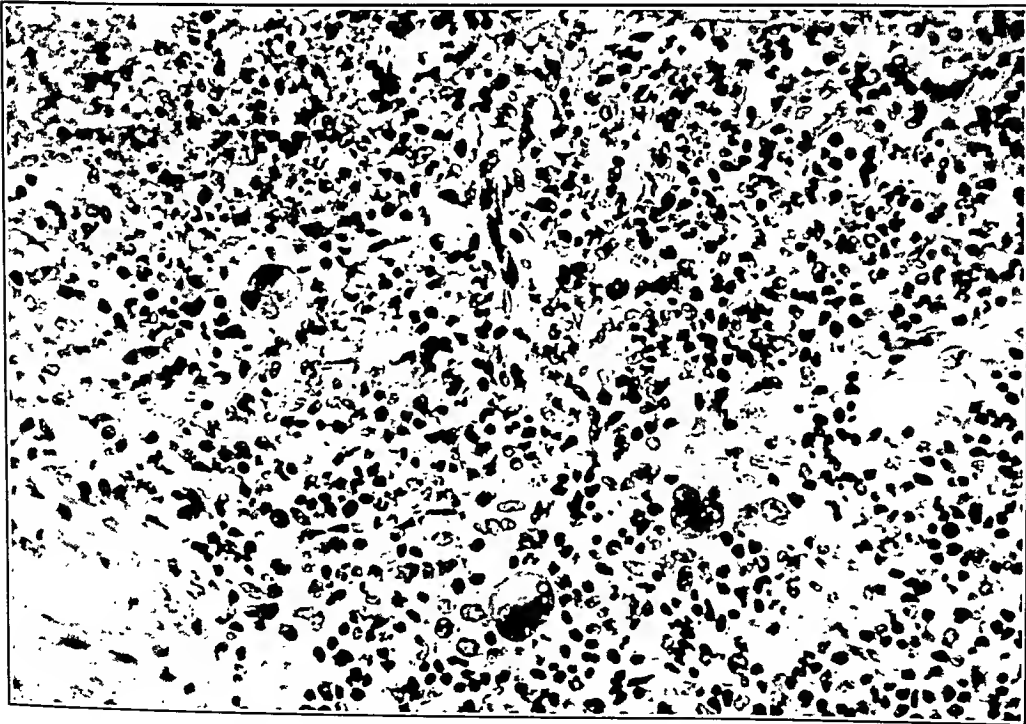
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### PLATE 81

- FIG. 1. Adult megakaryocyte showing normal numbers of granules in cytoplasm. From the femoral marrow of a case of idiopathic thrombocytopenic purpura hemorrhagica (35-480). Wright's megakaryocyte stain.  $\times 1425$ .
- FIG. 2. Spleen from same case (35-480) showing three adult megakaryocytes in sinuses. Phloxine-methylene blue stain.  $\times 450$ .



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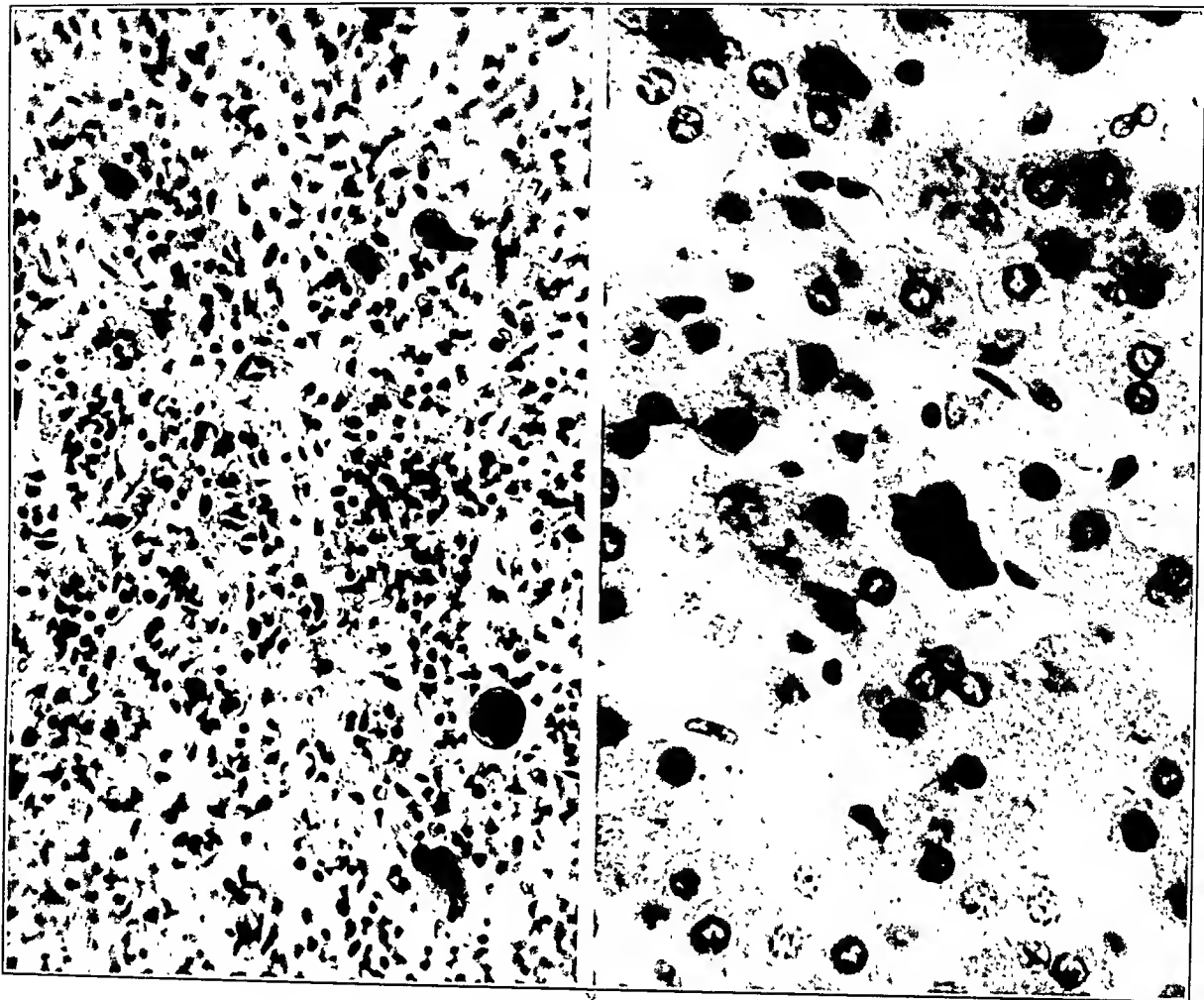
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PLATE 82

FIG. 3. Spleen from another case of idiopathic thrombocytopenic purpura hemorrhagica (35-412) showing five megakaryocytes in sinuses. The only degenerated form shows beginning inclusion of a leukocyte. Phloxine-methylene blue stain.  $\times 450$ .

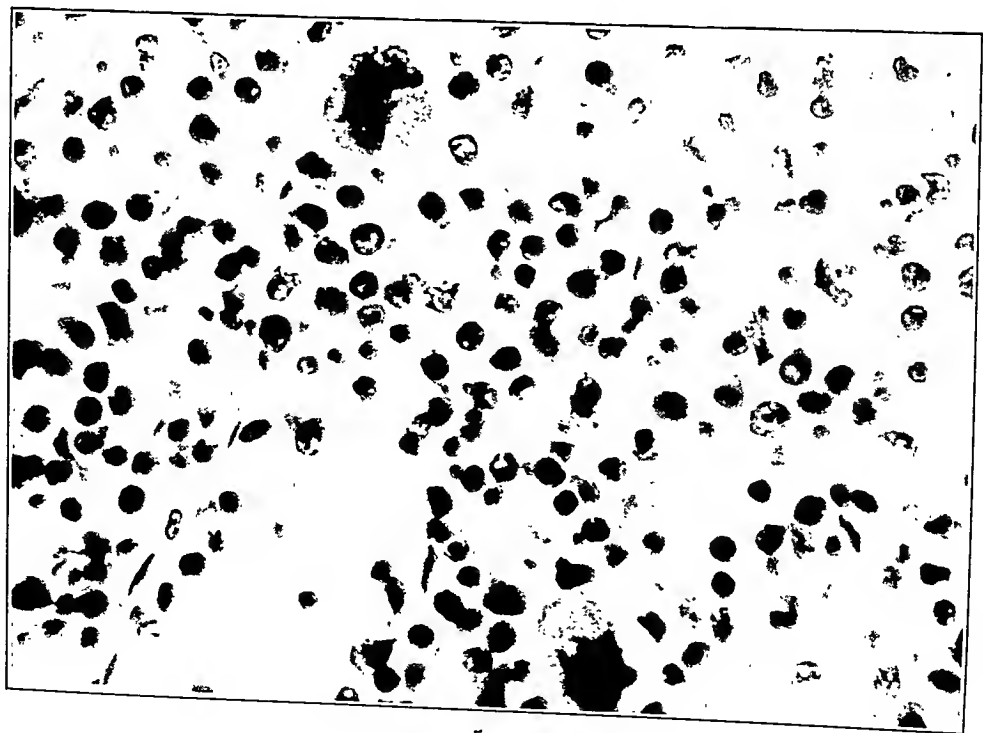
FIG. 4. Liver (35-480) showing an adult megakaryocyte in sinusoid. Phloxine-methylene blue stain.  $\times 930$ .

FIG. 5. Retroaortic lymph node (35-412) showing two megakaryocytes in sinuses. Phloxine-methylene blue stain.  $\times 930$ .



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Nickerson and Sunderland

Idiopathic Thrombocytopenic Purpura Hemorrhagica



## A NEW METHOD FOR THE RAPID STAINING OF MYELIN SHEATHS \*

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The various methods that have so far been devised for staining the myelin sheaths of nerve fibers suffer from one of two disadvantages, either an undue complexity of the method or too great a length of time necessary for its execution. Weigert's methods,<sup>1,2</sup> or any of the several modifications such as those of Pal<sup>3</sup> and Kultschitzky,<sup>4,5</sup> necessitate long periods of mordanting the entire block of tissue before embedding in a solution of either copper or chromium salts, or both. Preparation of the sections by these methods is so time consuming that a period of weeks or months must elapse before they are ready for study. Furthermore, these methods not only do not stain the cells, but the process of mordanting the blocks of tissue so alters the cellular elements that poor results are obtained when an attempt is made to stain the cells by other methods.

The iron hematoxylin method devised by Heidenhain<sup>6</sup> for staining tissues in general has been adapted to the staining of myelin sheaths by several investigators including Loyez,<sup>7</sup> Spielmeyer,<sup>8</sup> Morgan,<sup>9</sup> Weil,<sup>10</sup> and Clark and Ward.<sup>11</sup> While all of these procedures require much less time than Weigert's original method, or its modifications, none of them combines the qualities of reliability, simplicity and rapidity to such a degree as the method described in this report.

The method described herein may be performed in from  $\frac{1}{2}$  to 1 hour, depending on the thickness of the sections. It is applicable to normal or pathological material and works well on frozen, paraffin or celloidin sections. It has the further advantage that only the sections to be stained need be mordanted so that sections adjacent to the myelin stained ones may be stained by other methods. In addition, the chromophilic substance may be stained with excellent results in the section that has been stained for myelin sheaths. The myelin is stained a deep blue and the background remains either colorless or

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slightly tinted, depending on the degree of differentiation. The nuclei of all the cellular elements are stained in such a manner that the nuclear structure is clearly shown and it is therefore possible to differentiate the various types of glial cells from each other and from the nerve cells. The chromophilic substance may be stained in the same section by the use of neutral red, thionin or a similar stain.

### DIRECTIONS

1. Mordant sections for 15 minutes in a 4 per cent aqueous solution of ferric ammonium sulphate ( $\text{FeNH}_4(\text{SO}_4)_2$ ) prepared from violet crystals.

2. Rinse in 70 per cent alcohol to remove excess of mordant.

3. Place sections in a solution of 1 per cent hematoxylin containing 2 to 3 per cent glacial acetic acid by volume. (The 1 per cent hematoxylin solution is made by the addition of distilled water to a 10 per cent stock solution of hematoxylin in absolute alcohol.) The solution containing the sections is kept at or near  $55^\circ\text{C}$ . The sections at first stain deeply, then differentiate, and the depth of staining of the myelin sheaths depends on the time the sections are left in the staining-differentiating solution. Usually between 30 and 60 minutes are required but thin sections may require less time.

4. Place in a half saturated solution of lithium carbonate for 5 to 10 minutes.

5. Rinse in tap water.

6. Counterstain or stain for chromophilic substance if desired.

7. Dehydrate in alcohol, clear in xylol and mount in balsam.

### COMMENT

This method gives excellent results with paraffin, celloidin or frozen sections after a number of fixatives, including formalin, alcohol-formalin, and Bouin's fluid. Sections of brain stem which had been preserved in formalin for 5 years stained well. Fixatives containing copper or chromium salts, *e.g.* Zenker's fluid, are not applicable to this method since differentiation will not occur after their use.

Frozen sections can be cut directly from the fixative and stored either in 10 per cent formalin or in 70 per cent alcohol, from which they may be taken and placed directly in the mordant. Paraffin

sections can be stained mounted or unmounted, as desired, but it is necessary to remove the paraffin before the method is applied. In the case of celloidin sections the celloidin need not be removed for no stain is left in it at the completion of the process. On the other hand, if it is so desired, the celloidin may be removed without adverse effects, either before or after the staining process is completed.

The 4 per cent ferric ammonium sulphate mordant should be made from violet crystals and the solution can be used repeatedly as long as it remains clear and without a precipitate. Sections may be placed in the mordant directly from 70 per cent or 80 per cent alcohol, in which they have been stored, or from 10 per cent formalin solution or water. A period of 15 minutes in the mordant is ample; a longer time does no harm and, when convenient, a group of sections may be mordanted overnight and the process completed the next day. Sections that have been mordanted for 2 weeks have been successfully stained. After mordanting the sections are rinsed in 70 per cent alcohol in order to remove the excess of the ferric ammonium sulphate solution. Alcohol is used in preference to water because of the tendency of the water to remove too much of the mordant from the tissue.

The staining solution used in this procedure is unique in that the sections not only stain but also differentiate in the same solution. The staining-differentiating solution is prepared from a 10 per cent stock solution of hematoxylin in absolute alcohol. This solution does not require ripening and it may be used immediately after preparation. Ripening, on the other hand, does not affect the quality of the results. In order to avoid overripening about a month's supply is usually made up in advance and kept tightly corked. Just before using, the required amount of the 10 per cent stock solution is diluted to 1 per cent by the addition of distilled water and enough glacial acetic acid is added so that the solution contains 2 to 3 per cent glacial acetic acid by volume. This gives a sufficiently rapid differentiation in nearly all instances, but if a more rapid differentiation is desired the solution may be made up to contain as much as 8 per cent glacial acetic acid without any apparent harm to the sections. In every instance it is important that the sections be observed frequently in order to prevent differentiation from proceeding too far. A convenient way to handle the sections is to place them in the stain in Petri dishes on a slide warming table. Care



should be taken to make certain that the sections are entirely covered by the staining-differentiating solution and do not lie upon one another, otherwise an irregular differentiation occurs. When the section has been differentiated to the desired degree it is placed in a half saturated solution of lithium carbonate for about 5 minutes. In the lithium carbonate solution not only is the differentiation stopped but the hematoxylin that has not been fixed in the tissues diffuses into the solution. The section is then placed in tap water for a few minutes, dehydrated in graded alcohols and mounted in neutral balsam. If it is necessary to keep stained sections a day or two before mounting they should be placed in tap water since storage in alcohol causes the stain to fade slowly.

Occasionally it may be found necessary to interrupt the process of differentiation before it has been completed. Under such circumstances the section may be taken from the staining-differentiating solution, placed for 5 minutes in a half saturated solution of lithium carbonate and then transferred to tap water where it may be kept for as long as several days. When convenient the differentiation may be completed by placing the section in the staining-differentiating solution at 55° C. If for any reason the differentiation has been allowed to proceed too far, the section may again be mordanted and the staining-differentiating process repeated.

The advantage this method offers in making possible the staining of sections for myelin sheaths and the adjacent sections for other structures is further enhanced by the fact that a cell stain may be used on the myelin stained sections. Although the cytoplasm of the nerve cells is tinted and the chromophilic granules are usually faintly discernible in the sections, they are not stained well enough for careful study. If, therefore, it is desired to study the chromophilic substance in the myelin stained sections they can be stained in the usual manner by any of the chromophilic stains such as thionin, toluidine blue or neutral red. We have found that a 1 per cent aqueous solution of neutral red not only stains the chromophilic granules well but also affords a good contrast stain.

In addition to staining the myelin sheaths this procedure affords a nuclear stain of such excellence that details of nuclear structure are clearly shown and differentiation of cell types is possible on this basis. All of the cells show a more or less distinct nuclear membrane. The large nucleus of the nerve cell possesses a conspicuous black

staining nucleolus and an extremely delicate, net-like arrangement of nuclear chromatin. The nucleus of the astrocyte, usually about the size of that seen in the small nerve cells, possesses no such large and conspicuous nucleolus but usually contains a number of small deeply stained granules, two or three of which are decidedly larger than the others. The nuclei of the oligodendroglia appear smaller than either of the preceding and contain several relatively large, deeply staining, irregularly shaped chromatin masses scattered throughout the nucleoplasm. The microglia possess the smallest and most elongated nuclei of all the glial cells and have many deeply stained chromatin granules within the nucleoplasm. Red blood cells stain black, a characteristic that renders areas of vascular congestion or extravasation easily recognizable microscopically. The nuclear structure of endothelium, smooth muscle and connective tissue is clearly shown.

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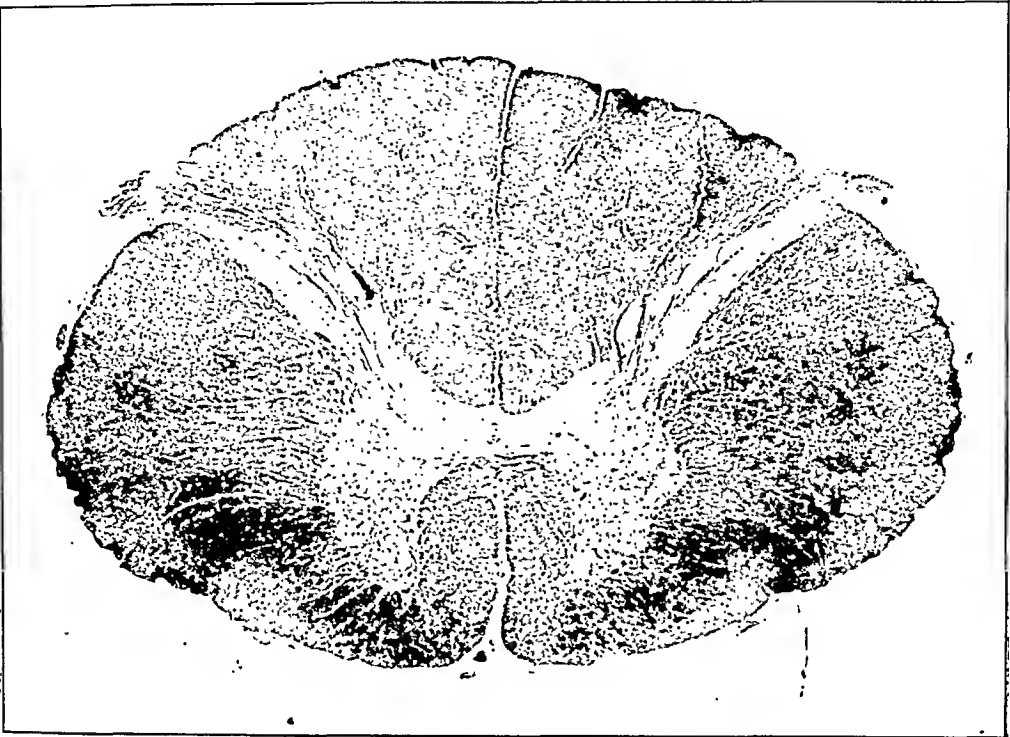
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## DESCRIPTION OF PLATES

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### PLATE 83

- FIG. 1. Human spinal cord stained by the new method showing the sharp demarcation between the white and gray matter and the amyelinic zone of the entering dorsal root. Formalin fixation, celloidin section, 20  $\mu$ .  $\times 10$ .
- FIG. 2. A portion of the dorsal columns from Fig. 1 showing the definitive staining of the myelin sheaths. Formalin fixation, celloidin section, 20  $\mu$ .  $\times 500$ .
- FIGS. 3, 4, 5 and 6. Nuclei of astrocyte, oligodendroglia, microglia and nerve cell from a myelin stained section of the cerebral cortex of the dog. Formalin fixation, celloidin section, 20  $\mu$ .  $\times 1500$ .



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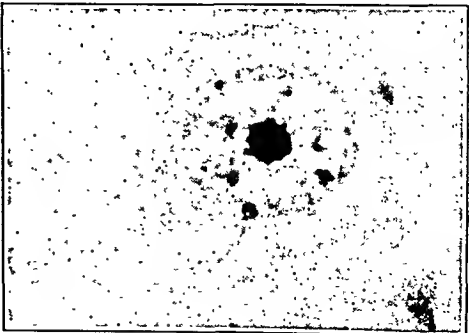
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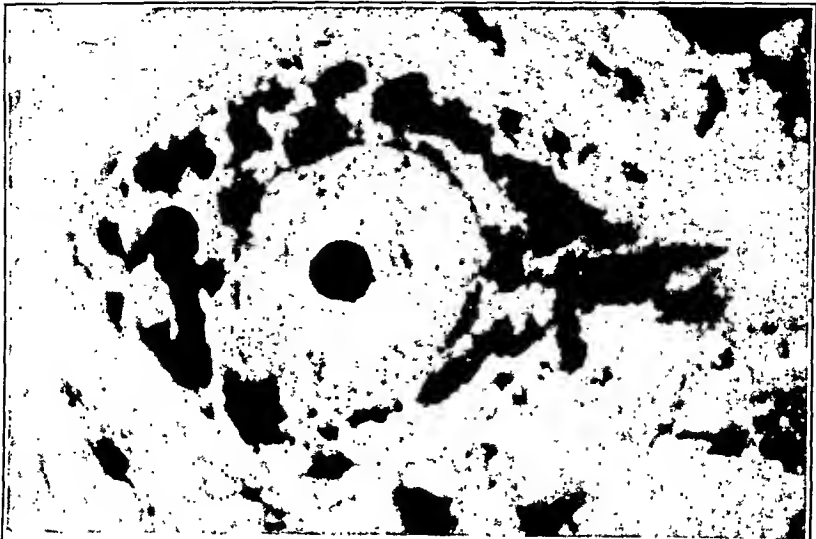
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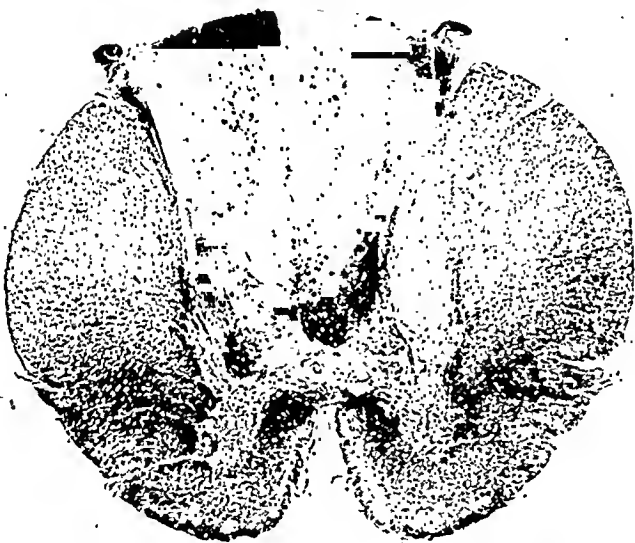
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PLATE 84

- FIG. 7. Nerve cell from a section of normal spinal cord stained with neutral red after the myelin sheath stain. The chromophilic granules have retained their normal arrangement and ability to stain. Formalin fixation, celloidin section,  $20\ \mu$ .  $\times 1500$ .
- FIG. 8. Spinal cord from a case of myelitis. Myelin sheath stain. Formalin fixation, paraffin section,  $20\ \mu$ .  $\times 10$ .
- FIG. 9. Section through the medulla oblongata from a case of multiple sclerosis. The demyelinated plaques are clearly defined by this staining method. Formalin fixation, celloidin section,  $20\ \mu$ .  $\times 5$ .



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## THE PATHOLOGY AND PATHOGENESIS OF CLINICAL ACUTE NEPHRITIS \*

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In this publication clinical acute nephritis includes all cases of acute renal disease that exhibit a definite impairment of renal function indicated by retention of nitrogenous products, decreased ability to excrete phenolsulphonaphthalein, inability to form a concentrated urine, loss of large amounts of protein in the urine, bleeding from the parenchyma of the kidney and severe oliguria or anuria. All forms of benign albuminuria, including febrile, are excluded since these show only trivial alterations in renal structure and function. Acute pyelonephritis and acute obstructive lesions of the urinary tract are also omitted from this discussion.

In the literature the various anatomical types of acute nephritis are not often clearly distinguished and for this reason it is difficult to compare the observations of different investigators. Many writers discuss acute nephritis as a clinical entity and assume that the underlying pathological process in the entire group varies only in intensity and not in character. Other authors distinguish nephritis and nephrosis and interpret nephrosis as tubular disease.

The 110 cases of acute nephritis which were available for study were subdivided into groups in accordance with the structural changes in the kidneys.

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## I. ACUTE GLOMERULONEPHRITIS

(A) Acute proliferative glomerulonephritis	
(a) Uncomplicated type	31 cases
(b) Associated with another disease	20 "
(B) Thrombosis of glomerular capillaries	1 "
(C) Exudative type	3 "
(D) Thrombosis of afferent arterioles	2 "
(E) Embolic type with uremia	
(a) With endocarditis	2 "
(b) Without endocarditis	5 "
(F) Hemorrhagic type with tubular obstruction	11 "
(G) Acute lipoid nephrosis	4 "
	<hr/>
Total glomerulonephritis	79 cases

## II. VASCULAR LESIONS

(A) Thrombosis of the renal arteries	1 case
(B) Widespread thrombosis of small arteries	1 "
(C) Polyarteritis nodosa	1 "

## III. INTERSTITIAL LESIONS

(A) Acute interstitial nephritis	1 "
(B) Acute lymphatic leukemia	1 "

## IV. TUBULAR DISEASES OF THE KIDNEYS

(A) Pure tubular degeneration	
(a) Mercuric chloride poisoning	7 cases
(b) Other types	2 "
(B) Obstructive tubular disease from blood trans- fusion	3 "

## V. EXTRARENAL UREMIA

(A) With renal injury	8 "
(B) Without renal injury	7 "

## I. ACUTE GLOMERULONEPHRITIS

(A) *Acute Proliferative Glomerulonephritis*  
(Table I, Types 1a and 1b)

This variety of acute nephritis is characterized histologically by increase in the number and size of the endothelial cells of the glomerular capillaries with subsequent partial or complete obstruc-

tion of the capillary lumens. It is of more interest than other forms since more clinical cases belong in this group and nearly all chronic glomerulonephritis originates from this type.

In a previous publication (Bell, 1936) the subclinical forms of acute proliferative glomerulonephritis were described. It was pointed out that various acute infections, notably subacute bacterial endocarditis and puerperal sepsis, cause a more or less marked increase in the number and size of the glomerular endothelial cells. There is no sharp histological distinction between subclinical and clinical glomerulonephritis, but, in general, clinical symptoms appear as soon as hyaline fibers have formed in the capillaries. In this discussion a case is considered clinical when the glomerular lesions are severe, and in most instances clinical evidence of acute nephritis was also recorded.

There were 31 fairly typical examples of clinical acute proliferative glomerulonephritis (Table I, Type 1a), and there were 20 cases (Type 1b) in which the symptoms of nephritis were overshadowed by some major illness. The structural changes in the kidneys were identical in the two subgroups.

The kidneys are usually enlarged; in 31 of 37 adults the combined weight was over 300 gm., and in 13 cases over 500 gm. On section the surfaces are invariably pale and cloudy. On the basis of the macroscopic appearance alone a diagnosis of acute nephritis cannot be made with certainty since many kidneys with simple cloudy swelling present similar features.

Microscopically under low magnification the glomeruli appear very cellular and few or no erythrocytes are to be seen. There is obviously a great increase in the number of nuclei and the capillaries are occluded with nucleated cells. It is noteworthy that the glomeruli are not always greatly enlarged; in fully one-third of the cases there is only a moderate increase in their diameters. There is usually a high degree of endothelial proliferation in the capillaries of a great majority of the glomeruli, but there are always a few glomeruli with only a slight endothelial increase. In some of the cases where death was due to edema of the lungs (Nos. 20, 23, 26), at an early stage of the disease the endothelial proliferation is less pronounced than in those where death was due to uremia, but there is no close correlation between the duration of symptoms and the degree of endothelial increase. Apparently the process proceeds more rapidly in

some instances than in others. When the glomerular tufts are compressed by large epithelial crescents (Nos. 27, 28, 29, 50), the endothelial proliferation is much less pronounced.

Hyaline fibers can always be seen in sections stained by the Mallory-Heidenhain stain (azocarmine) except in tufts compressed by large epithelial crescents. These fibers are at first few in number and delicate, but later they usually become more numerous and coarser. They are apparently derived from the capillary basement membrane, especially the part forming the deep surface of the capillary wall. This feature will be discussed more fully later.

The capillary basement membrane does not often show a uniform diffuse thickening, as seen in eclampsia and in many cases of lipoid nephrosis. When the membrane is thickened it is usually split into two or three layers (No. 7) and does not appear as a single heavy band, as in the diseases just mentioned. The changes in the basement membrane will be described in more detail in the discussion of pathogenesis.

Polymorphonuclear leukocytes are found in increased numbers in 34 of the 51 cases. They were searched for only in hematoxylin-eosin preparations; the oxidase reaction would probably have demonstrated an increase in every instance. The prominence of the leukocytes is indicated in the table by numerals, "0" being the number found in normal glomeruli. When the leukocytes were the chief cause of the capillary obstruction (Grade 4) the kidney was classified as an exudative type of nephritis (Type 3). There is evidently an exudative feature in most of the cases of the proliferative type, but obstruction is due almost entirely to endothelial cells.

There is thrombosis of occasional afferent glomerular arterioles in 11 of the 51 cases. When this was the outstanding alteration responsible for renal insufficiency the kidneys were classified in a special group (Type 4).

Epithelial crescents are present in 18 of the 51 cases, and sometimes they are more important than endothelial proliferation in causing obstruction of the glomerular circulation (Nos. 27, 28, 29, 50). Large crescents compress the glomerular tufts to such a degree that no blood can pass through the capillaries. Nephritis in which epithelial crescents are unusually prominent is sometimes called "extracapillary" glomerulonephritis, but the extracapillary lesions

are so intermingled with the intracapillary proliferative forms that they hardly constitute a distinct histological type of nephritis.

It is clear that there are relatively few cases of pure endothelial proliferation; there are commonly some polymorphonuclear leukocytes, epithelial crescents or thrombosed arterioles. The changes in the capillary basement membrane will be described presently.

*Histogenesis of the Glomerular Lesions:* In a previous publication the subclinical stages of glomerulonephritis have been described. In infectious diseases, especially bacterial endocarditis and puerperal sepsis, there is often a rather marked endothelial increase which differs from clinical acute glomerulonephritis only in intensity and in the absence of hyaline fibers. There are gradual transitions between the subclinical and the clinical stages. Apparently symptoms and signs of nephritis usually do not appear until glomerular obstruction is pronounced.

The less advanced lesions in clinical cases correspond entirely to those of the subclinical stage previously described. The capillaries are partially filled with mononuclear cells attached to the basement membrane. These cells have been interpreted by nearly all investigators as of endothelial origin. Recently, however, MacCallum has sponsored the view that they arise from connective tissue cells between the capillaries. MacCallum's theory is based largely on his observation that the capillary basement membrane surrounds the minute lobules of the glomerulus but does not form a complete investment for each capillary. However, in a normal glomerulus, appropriately fixed and stained, the basement membrane is easily seen as a complete layer around each capillary; it is just as prominent on the inner walls of capillaries within the lobule as it is at the surface of the lobule. In the subclinical stages of glomerulonephritis the basement membranes within the lobule are also readily seen and there is no doubt that the cells in question lie within the capillaries (see *Am. J. Path.*, 1936, 12, 801, Figures 3, 5 and 6). However, after a glomerulonephritis is established the layer of basement membrane on the deep surface of the capillary splits into fragments, which form hyaline fibers, and a continuous basement membrane is no longer seen except at the surface of the lobule (see *Am. J. Path.*, 1936, 12, Plate 132, Figs. 7 and 8). A study limited to well developed examples of glomerulonephritis might lead one to the false conclusion that the basement membrane is absent within the lobule and that

intercapillary fibroblasts give rise to the cells and fibers that obstruct the capillaries.

An early stage of clinical glomerulonephritis is shown in Figure 1. The small lobule shown in the drawing is only a little more advanced than the subclinical stage (compare *Am. J. Path.*, 1936, 12, Plate 131, Fig. 6). The capillaries are well filled with large endothelial cells and only an occasional erythrocyte is to be seen. The basement membrane of the outer wall of the capillaries is largely intact but the layer forming the inner capillary wall is no longer even and continuous, as it is in the normal and the subclinical stages. It shows several interruptions and there are a few delicate blue stained fibrils apparently branching off from this layer and penetrating the cytoplasmic mass. This is an early stage in the formation of hyaline fibers and they appear to have been split off from the inner basement membrane.

In Figure 2 a later stage is illustrated. The basement membrane of the outer surface of the capillaries is intact but the inner layers are split into numerous hyaline fibers. The individual capillaries can no longer be distinguished. Unless the splitting of the inner basement membrane is followed from its earliest stages it may appear that there is no basement membrane covering the deep surface of the capillaries and that the hyaline fibers are derived from intercapillary fibroblasts. But when successive stages of the process are studied it is readily seen that the hyaline fibers are split off from the inner basement membranes. There are no intercapillary cells in these small peripheral lobules, except occasional epithelial cells that extend inwards from the surface layer.

When the reaction has progressed to the stage shown in Figure 2 the inner walls of the capillaries can no longer be identified, since the capillaries have become fused on their deep surfaces. The central portion of the lobule is now composed of endothelial cells and hyaline fibers. In the lobule shown in Figure 2 all the capillary lumens have been obliterated, but often portions of the lumens persist at the periphery. This appearance may give the impression of an intercapillary accumulation of cells and fibers, but it is actually due to fusion of the adjacent capillaries and splitting of the inner basement membranes.

When portions of the capillary lumens at the periphery of the lobule persist and enlarge the fibers are pushed toward the center

of the lobule where they fuse into a homogeneous mass which gives the impression of intercapillary material. In some cases of chronic glomerulonephritis the central hyaline masses in the lobules are conspicuous and have been interpreted as "intercapillary glomerulonephritis." However, if one follows glomerular lesions from their earliest stages it becomes clear that all so-called intercapillary lesions are due to splitting and duplication of the inner basement membranes and subsequent compression of them in the center of the lobule. This feature will be described more fully in a subsequent paper.

When the capillaries of a glomerulus are completely filled with endothelial cells and hyaline fibers the glomerulus soon undergoes hyaline degeneration, if the patient survives, but this does not occur in the acute stage. When the capillaries are not completely closed circulation continues in the periphery of each lobule.

In acute proliferative glomerulonephritis terminating in uremia the glomerular lesions have commonly progressed no farther than the stage shown in Figure 2. In a subsequent paper these lesions will be followed into the chronic stage. It will be shown that glomeruli with completely closed capillaries soon become hyaline. When the capillaries are incompletely closed the force of the blood flow seems to open them to some extent, the hyaline fibers and cells being pushed toward the walls of the capillaries. Nearly all persistent glomeruli in chronic cases show thick basement membranes.

It is probable that lesions, such as shown in Figure 1, may terminate in recovery. We do not know the glomerular structure in instances of recovery, but presumably the alterations are much less intense than in those of death from uremia.

### *(B) Thrombosis of Glomerular Capillaries (Table I, Type 2)*

This patient (No. 52) presented the clinical features of septicemia rather than of nephritis although she had albuminuria and hematuria. The glomeruli show no exudative or proliferative changes but a large proportion of the glomerular capillaries is occluded by hyaline thrombi (Fig. 3). The thrombi are similar to the acute thromboses of bacterial endocarditis, but they do not have a focal distribution. There was no endocarditis in this case. In Case No. 31 there are also many thrombosed capillaries but the chief lesion is proliferative glomerulitis.

(C) *Exudative Type (Table I, Type 3)*

This form of nephritis is usually associated with a severe staphylococcic infection which may overshadow the renal symptoms. In 1 case (No. 55) renal insufficiency was demonstrated, and in another (No. 54) a severe oliguria was present. The glomerular capillaries are all distended with polymorphonuclear leukocytes, and these cells are also found in large numbers in the tubules. This form of glomerulitis presumably does not pass over into a chronic type. Staphylococci are believed to be the causative agents.

(D) *Thrombosis of Afferent Arterioles (Table I, Type 4)*

One of the patients of this group (No. 56) was a young infant with congenital syphilis, complicated in the terminal stages by pneumonia and pneumococcic peritonitis; the other patient (No. 57) was diagnosed clinically as suffering from acute nephritis and died in uremia. In both cases a large majority of the afferent glomerular arterioles are occluded by fresh hyaline thrombi. Some of the glomeruli are infarcted but they show no other striking changes. Juhel-Rénoy, in 1886, reported a case of this type in a girl 16 years of age. The patient developed anuria 2 days after the appearance of a scarlet fever eruption, which persisted until death 5 days later. Extensive thrombosis of glomerular arterioles was found at post-mortem.

(E) *Embolic Type With Uremia (Table I, Type 5)*

It is well known that bacterial endocarditis may terminate in uremia. Usually in such cases a diffuse proliferative glomerulonephritis is found at postmortem, but sometimes one finds numerous massive lesions of the embolic type which have blocked nearly all of the glomeruli (Fig. 4). Lesions of embolic type may occur in the absence of endocarditis; in fact endocarditis was present in only 2 of the 7 cases of this type, and we have 2 cases of embolic glomerulonephritis in which endocarditis was present only on the valves of the right heart. Patients Nos. 58 and 59 were diagnosed clinically as suffering from endocarditis and it was recognized that the former died of uremia. The other 5 cases were interpreted clinically as acute glomerulonephritis. In a previous publication (1932) I have described the structure of these so-called embolic lesions; they are

embolic in the sense that they are caused by the lodgment of bacteria but not in the sense that they cause infarction of parts of the glomerulus. There are two types of embolic lesions: the acute thrombotic form and the fibrous lesion. The thrombotic lesions are merely capillary thromboses, usually with necrosis of some of the capillary loops. A few thrombosed capillaries are frequently seen in the proliferative type of nephritis and in 3 cases (Nos. 31, 51 and 52) they were present in enormous numbers. The fibrous lesions are caused by focal splitting and thickening of capillary basement membranes. Contrary to the common belief the fibrous lesions do not develop from the thrombotic ones. In the cases listed (Nos. 58 to 64) the lesions are numerous and large, and both thrombotic and fibrous types are present. Probably these lesions are produced by bacteria circulating in the blood stream, which lodge in the glomerular capillaries.

*(F) Hemorrhagic Type With Tubular Obstruction (Table I, Type 6)*

This form of nephritis is characterized clinically by a severe infection of some kind and a pronounced hematuria. Often there is a bacteriemia. In 6 of the 7 cases in which blood urea nitrogen was determined it was found markedly increased. One patient had anuria.

The glomerular capillaries in these kidneys show only slight to moderate endothelial increase and there is evidently very little obstruction of their lumens. A great many of the capsular spaces and tubules are, however, distended with blood (Fig. 5). The blood evidently escapes from minute ruptures in the glomerular capillaries. In many of the tubules the red cells appear as compact masses, often with threads of fibrin between them; in other tubules many of the erythrocytes are laked, and amorphous masses of hemoglobin are seen. In the collecting tubules especially there are numerous loose casts composed of hemoglobin, erythrocytes and albumin (Fig. 6). The cortical tubules are uniformly dilated, presumably because of the numerous casts. The tubular epithelium often shows hyaline granular degeneration but very few cells are necrotic. The tubular obstruction is not unlike that seen in the transfusion kidney to be described presently, except that the obstruction is usually due to erythrocytes rather than to hemoglobin.

It appears that bacterial toxins injure the glomerular capillaries causing them to rupture and liberate large quantities of blood into



TABLE I  
Group I. *Acute Glomerulonephritis*  
Type I a. *Proliferative Type — Uncomplicated by Other Diseases*

Serial No.	Autopsy No.	Age	Sex	Duration	Albuminuria	Hematuria	Edema	Blood pressure	Blood urea nitrogen mg. per 100 cc.	Endothelium	Hyaline fibers	Basement membrane	Leukocytes	Thrombosis of arterioles	Crescents	Weight of kidneys	Etiology and other data	Chronic passive congestion of liver
1	13-140	yr. 12	F	3 mos.	3	0	4	?	?	3	1	0	1-	0	0	Normal	A severe cold followed by pneumonia. Pneumococcic peritonitis	1
2	15-65	55	M	6 wks.	2	+	2	?	?	3	1	0	1-	0	0	Normal	No preceding infection	?
3	15-230	34	M	?	?	?	0	?	?	2	1	0	1-	0	0	490	No history	?
4	17-176	66	F	1 mo.	3	0	1	?	72 (2 wks.)	3	1	0	1	1-	0	300	A cold followed by bronchitis	0
5	19-152	12	M	5 wks.	3	0	2	132/94	?	3	2	0	1-	0	8	245	Followed smallpox	1
6	21-108	51	M	1+ mo.	?	?	2	228/110	?	3	3	0	0	1	0	630	No preceding infection	0
7	22-468	35	M	2 mos.	?	+	2	100/?	?	3	1	3	1-	0	2	365	Followed pneumonia. Empyema. Multilayered basement membrane	2



TABLE I. GROUP I. TYPE I a—Continued

Serial No.	Autopsy No.	Age	Sex	Duration	Albuminuria	Hematuria	Edema	Blood pressure	Blood urea nitrogen mg. per 100 cc. (8 days)	Endothelium	Hyaline fibers	Basement membrane	Leukocytes	Thrombosis of arterioles	Crescents	Weight of kidneys	Etiology and other data	Chronic passive congestion of liver
17	29-1799	62 yrs.	M	1 mo.	4	0	1	190/105	190 (8 days)	2	2	0	0	0	1	550 gm.	Influenza 2 wks. before onset	1—
18	30-526	61	M	3 wks.	3	0	0	?	?	3	1	0	2	0	0	550	Onset 1 wk. after a sore throat. Oliguria. Anuria	1
19	30-919	68	F	1+ mo.	?	?	0	190/80	?	3	1	0	2	1—	2	385	A cold 3 mos. before death. Pyuria	1
20	32-1642	7	F	6 days	?	?	1	?	?	2	1	0	1—	0	0	148	Sore throat 3 wks. before death. Death from edema of lungs	0
21	32-1706	7	M	3 days	4	0	1	?	?	3	1	0	1	0	0	150	Developed impetigo contagiosa 3 wks. before death. Severe edema of lungs	2
22	33-302	51	M	16 days	3	0	3	164/80	100 (4 days)	3	1	0	1	0	0	420	Sore throat 3 wks. before death	1
23	33-469	7	M	?	?	+	0	?	?	2	1	0	1—	0	0	?	Scarlet fever 6 wks. before death. Death from edema of lungs	1

24	33-1598	14	F	9 days	?	?	?	1-	160/115	?	3	1	0	2	0	0	170	Onset with sore throat and bronchitis	1
25	35-638	35	M	2 wks.	4	0	3	146/90		35 (1 hr.)	3	3	0	1	0	0	460	No preceding infection. Death from edema of larynx and lungs	?
26	35-793	35	F	6 wks.	2	?	2	?	?	70	2	1	3	1	0	1	?	Edema of lungs	?
27	18-62	27	M	5 wks.	3	0	1	?	?	?	1	0	0	3	2	4	290 (one)	Nephrectomy followed by suppuration of wound. Pyuria	0
28	30-602	1	M	5 wks.	4	0	0	?	?	?	1	0	0	0	0	4	200	Pharyngitis. Otitis media	0
29	32-1434	32	M	5 wks.	?	?	3	180/110		NPN 159 (5 days)	2	0	2	0	0	4	535	Cold followed by bronchitis 9 wks. before death	0
30	35-752	44	F	10 wks.	4	0	1	150/60		?	3	3	2	2	0	0	270	Hydrothorax (3). Ascites. PSP 30% (2 wks.). Ruptured aneurysm of mesenteric artery	0
31	24-288	40	F	1 mo.	2	0	1	180/110		32 (3 wks.)	3	1	0	0	0	1	490	Numerous thromboses in glomerular capillaries	0

TABLE I. GROUP I — Continued  
Type I b. Proliferative Type — Associated With Other Diseases

Serial No.	Autopsy No.	Age	Sex	Duration	Albuminuria	Hematuria	Edema	Blood pressure	Blood urea nitrogen mg. per 100 cc.	Endothelium	Hyaline fibers	Basement membrane	Leukocytes	Thrombosis of arterioles	Crescents	Weight of kidneys	Etiology and other data
32	17-202	yrs. 25	M	?	3	?	4	140/80	?	3	3	0	1	0	0	gm. 515	Bacterial endocarditis
33	22-581	19	F	6 wks.	?	?	2	170/?	?	3	1	0	1	1—	0	350	Puerperal sepsis. <i>Strept. viridans</i> septicemia
34	18-122	30	M	?	3	0	3	?	?	2	1	0	1—	0	0	530	Bacterial endocarditis
35	32-910	11	F	3 wks.	1—	0	0	?	?	3	2	0	0	1—	0	210	Lupus erythematosus
36	36-71	40	F	?	3	0	1	132/78	86 (6 days)	2	1—	0	1	1	0	345	Organizing pneumonia
37	16-48	58	M	3 wks.	?	?	1	140/?	?	3	1	0	3	1	2	573	Diabetic gangrene. Arteriosclerosis
38	20-220	55	M	?	?	?	3	?	?	3	1	0	2	0	0	397	Alcoholism. Hypertension
39	25-360	69	M	10 days	4	0	1	?	?	3	1	0	1	1—	0	370	Began with a cold. Hypertension. Arteriosclerosis
40	29-456	78	M	3 wks.	?	?	2	215/110	131 (1 day)	3	3	0	0	0	3	260	Hypertension. Arteriosclerosis

41	32-782	60	M	?	2	0	1	265/142	34 (1 mo.)	3	3	0	1	0	1	450	Hypertension. Many capillary thrombi
42	24-580	68	M	?	3	0	3	200/115	41 (6 wks.)	2	1	0	0	1	0	275	Hypertension
43	32-798	62	M	3 mos.	1-	0	1	220/140	103 (1 day)	3	1	0	0	0	0	510	Hypertension with cardiac decompensation
44	33-64	74	M	?	1-	0	3	190/75	?	3	1	0	1	0	0	710	Hypertension. Pyonephrosis
45	31-1423	37	M	?	3	0	2	120/70	30 (3 mos.)	2	1	1	0	0	1	275	Old aortic valve defect with cardiac decompensation
46	30-259	78	M	5 mos.	?	+	2	130/78	158 (2 days)	3	1	0	2	0	3	200	Hypertrophy of prostate. Infected surgical wound
47	32-391	56	F	?	2	0	1	160/80	77 (1 day)	3	3	2	0	0	0	515	Cystitis. Hydro-nephrosis. Arteriosclerosis
48	32-619	13	M	3 wks.	2	0	1	120/74	78 (4 days)	3	2	0	1	0	0	400	Pulmonary tuberculosis. Began with a cold
49	35-1181	38	F	?	1-	0	0	90/60	?	3	3	0	1	0	0	400	Hypertension with cerebral hemorrhage
50	31-954	35	M	?	?	?	0	?	?	2	1	1	0	0	4	500	Bacterial endocarditis. No embolic lesions

TABLE I. GROUP I. TYPE I b — Continued

Serial No.	Autopsy No.	Age	Sex	Duration	Albuminuria	Hematuria	Edema	Blood pressure	Blood urea nitrogen	Endothelium	Hyaline fibers	Basement membrane	Leukocytes	Thrombosis of arterioles	Crescents	Weight of kidneys	Etiology and other data
51	32-255	62 yrs.	M	?	?	?	0	200/100	?	2	1	0	0	0	0	441 gm.	Hypertension. Extensive thrombosis of glomerular capillaries

Type 2. Thrombosis of Glomerular Capillaries

52	34-711	44	F	9 days	3	+	0	128/72	14 (5 days)	0	0	0	0	0	0	460	Began with upper respiratory infection. Septicemia. Widespread thrombosis of glomerular capillaries
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Type 3. Exudative Type

53	15-323	49	M	?	2	0	2	160/110	?	2	0	0	4	0	3	330	Aortic aneurysm
54	18-251	18	F	4 days	4	0	1-	?	?	1	0	0	4	0	0	540	Peritonillar abscess. Hemolytic streptococcus bacteremia. Severe oliguria
55	25-222	18	F	?	3	0	0	140/76	138 (1 wk.)	1	0	0	4	0	0	671	Pelvic abscess. Pyemia





TABLE I. GROUP I. TYPE 5 — Continued

Serial No.	Autopsy No.	Age	Sex	Duration	Albuminuria	Hematuria	Edema	Blood pressure	Blood urea nitrogen mg. per 100 cc. (8 days)	Endothelium	Hyaline fibers	Basement membrane	Leukocytes	Thrombosis of arterioles	Crescents	Weight of kidneys	Etiology and other data
63	22-363	23 yrs.	M	2 mos.	1	0	1	?		1	0	1	0	0	3	gm. 425	Extensive embolic lesions. No endocarditis
64	34-584	50	M	5 wks.	4	+	1	130/100	167 (3 days)	1	1	0	0	0	3	570	Extensive fibrous embolic lesions. No endocarditis

Type 6. Hemorrhagic Type

65	13-153	13	F	15 days	?	++	0	?	?	1	0	0	0	0	0	Large	Began with sore throat. Severe oliguria. Streptococcal peritonitis
66	19-23	31	F	6 wks.	?	++	0	?	?	1	0	0	1	0	0	440	Pneumococcal bacteremia and meningitis
67	23-737	15	F	2 mos.	?	++	1-	?	?	1	0	0	1	1-	0	410	Streptococcal bacteremia and peritonitis

68	26-209	9	F	3 wks.	?	++	1	?	58 (11 days)	1	0	0	0	0	1-	330	Followed scarlet fever. Otitis media. Streptococcic bacteremia
69	26-515	24	M	1 mo.	?	++	0	148/60	122 (6 days)	1	0	0	1	1-	1	Very large	Acute osteomyelitis. A few embolic lesions. No endocarditis
70	31-804	55	F	2 wks.	1	++	1	?	95 (1 day)	2	0	0	0	0	2	405	Enormous hemorrhage into tubules. A few embolic lesions. No endocarditis
71	33-235	56	F	?	3	++	1	166/92	87 (12 days)	2	0	0	1	1-	0	400	Hyperthyroidism
72	33-832	25	F	4 days	?	?	0	?	85 (2 days)	0	0	0	0	0	0	500	Puerperal sepsis. Tubules full of blood
73	35-1961	50	M	1 wk.	1-	+	0	170/100	?	1	0	0	0	0	0	398	Lobar pneumonia. Tubules full of blood
74	33-288	5	M	8 days	?	++	0	?	168 (2 days)	2	1-	0	0	2	0		Sore throat. Streptococcic peritonitis. Anuria
75	33-452	22	F	1 mo.	1	++	0	128/90	28 (2 days)	2	0	0	0	0	0	525	Followed a peritonillar abscess. Severe vomiting

TABLE I. GROUP I — Continued  
Type 7. Acute Lipoid Nephrosis

Serial No.	Autopsy No.	Age	Sex	Duration	Albuminuria	Hematuria	Edema	Blood pressure	Blood urea nitrogen mg. per 100 cc.	Endothelium	Hyaline fibers	Basement membrane	Leukocytes	Thrombosis of arterioles	Crescents	Weight of kidneys	Etiology and other data
76	34-179	4 yrs.	M	2 mos.	4	0	1	108/70	23.8 11.6	1	0	0	0	0	0	290 gm.	Began with conjunctivitis. Death from streptococcal peritonitis
77	35-114	58	M	4 mos.	4	0	3	140/90	15.9	0	0	2	0	0	0	270	Oliguria. Streptococcal peritonitis
78	35-654	36	M	5 wks.	4	0	3	?	?	0	0	0	0	0	0	365	NPN 38 mg. (2 wks.). Death due to accident
79	34-383	1½	M	6 wks.	4	0	3	?	?	1	0	0	0	0	0	300	Otitis media. Peritonitis. Streptococcal fatty tubules

0 = normal. The intensity of the various processes is indicated by numerals.  
Under urea nitrogen the time before death is indicated.

the tubules. The blood cells become impacted in some of the tubules and actual coagulation sometimes occurs. Hemoglobin casts are rare and may be due to hemoglobinemia or to laking of the red cells in the tubules. Although the uremia accompanying this type of nephritis is due to tubular obstruction the primary injury is glomerular and for this reason the disease is classified as glomerulonephritis.

The bleeding glomeruli show no lesions that one would expect to persist in a chronic form; there are only minute ruptures in the capillaries from which the blood escapes. It is believed that the benign renal hematurias that sometimes follow tonsillitis have similar glomerular lesions; the mild nature of the lesions would explain the rapid recovery.

#### (G) *Acute Lipoid Nephrosis (Table I, Type 7)*

Lipoid nephrosis is usually a chronic form of renal disease, but occasionally it terminates in death several weeks after the onset. Four cases of the acute form (Nos. 76 to 79) are included in this discussion since they represent a variety of acute nephritis. The patients presented the well known clinical features, *i.e.* massive albuminuria, marked edema (except in No. 76) and absence of renal insufficiency. In 3 cases death was due to streptococcic peritonitis. The glomeruli show practically no changes except in No. 77 in which there is a rather well marked thickening of the capillary basement membranes. Although the glomeruli are histologically normal in many of these acute cases, the basic disturbance is probably injury of the glomerular capillaries. This is indicated by the increased permeability of the capillaries to plasma proteins and by the fact that the chronic cases usually develop a well marked thickening of the capillary basement membranes. Lipoid nephrosis will be discussed more fully in a subsequent publication.

#### CLINICAL PHENOMENA OF ACUTE GLOMERULONEPHRITIS

The 79 cases of acute glomerulonephritis may be discussed as one clinical group although the glomerular lesions vary strikingly in the different types.

*Frequency:* Only 42 of the 79 cases were good clinical examples of acute glomerulonephritis; 37 cases were associated with another disease and the renal lesion was considered of secondary importance. These cases were collected from a careful study of about 25,000 post-

mortems. If we consider only the cases uncomplicated by another disease, in which the glomerular lesions were of such a nature that they might have progressed to chronic glomerulonephritis, there are 42 cases. In this same series of postmortems there were 120 deaths from chronic glomerulonephritis, from which it is obvious that death from glomerulonephritis is much more apt to occur in the chronic than in the acute stages of the disease. Since acute glomerulonephritis so often terminates in recovery it is no doubt seen much oftener in clinical practice than in the autopsy room.

*Age:* Seegal, Seegal and Lyttle analyzed the clinical records of 381 cases of acute glomerulonephritis from several hospitals, the diagnoses being made from the hospital records. They found that about 50 per cent of the cases occurred in the first decade, about 20 per cent in the second, about 15 per cent in the third, and small percentages in subsequent decades.

Our group of uncomplicated cases is too small for statistical analysis, but over half of them were over 30 years of age.

*Sex:* Seegal, Seegal and Lyttle found acute nephritis about twice as frequent in males as in females, but Murphy reported 51 males and 53 females.

*Duration:* The duration of symptoms referable to the kidneys is shown in Table I. This is difficult to determine accurately, especially in cases complicated by another disease. In the uncomplicated cases the onset is considered the time when the patient first noticed edema or hematuria, or realized that he was ill. However, there is often a continuous illness from the onset of the initial infection (sore throat, common cold, and so on) until the appearance of edema or hematuria. There are several cases in which one may be reasonably sure that the age of the renal lesion is less than 1 week. The duration of the renal disease depends on several factors: (a) death may be due largely or in part to the associated disease of which the nephritis is a terminal complication; (b) it may be hastened by the development of bacteriemia or peritonitis during the course of the nephritis; (c) it may occur early in the course of nephritis from edema of the lungs or larynx; and (d) it may be caused by uncomplicated renal insufficiency. The cases of short duration give opportunity for study of the early stages of the glomerular lesions.

*Albuminuria:* The diagnosis of acute glomerulonephritis cannot be made in the absence of albuminuria. In this series protein was

found in every case in which the urine was examined, and usually in large amounts (2 to 4+). In those instances in which only a trace, 1—, is recorded the patient had another disease and the last examination of the urine was usually made several days prior to death, before the nephritis had reached its maximum intensity. In the presence of hematuria, albumin in the urine has no additional significance. The amount of albumin in samples of urine often varies greatly from time to time and does not indicate accurately the extent of the renal damage. Protein escapes from the blood through injured glomerular capillaries, but the amount of leakage is not proportional to the visible structural changes; in fact protein cannot escape from capillaries unless they are open enough to allow the free passage of blood. The most severely damaged glomeruli have closed capillaries and do not transmit any albumin. The leakage of protein is greatest through the best, *i.e.* the most open capillaries. In some cases of acute lipoid nephrosis the capillaries show no visible alterations of structure yet they allow the passage of large quantities of protein.

In general, a marked albuminuria indicates nephritis of some form, but Murphy and coworkers found no relation between the degree of albuminuria and the subsequent course of acute glomerulonephritis. Albuminuria obviously depends on glomerular and not on tubular injury.

*Hematuria:* Hematuria is used to denote urine that contains sufficient blood to give it a red color, or at least a reddish tinge. In 11 cases (Type 6) hematuria was the most prominent clinical evidence of renal injury, and in 11 other cases it was present but less conspicuous. Red cells were found in the centrifuged urine in most instances when they were looked for, but often they were no more numerous than in cases of passive congestion of the kidneys. Murphy found gross hematuria in 36 of 94 cases, and erythrocytes were found in the sediment at some time in every case. In some cases of hypertension with acute renal insufficiency a definite hematuria is present and erythrocytes are also found in the urine in simple passive congestion. It seems therefore that "glomerulonephritis" is a more accurate term than "hemorrhagic nephritis," which some writers employ.

The erythrocytes escape from open glomerular capillaries, not from those plugged with endothelial cells or leukocytes. In microscopic sections one sees that the erythrocytes have escaped from

capillaries that appear normal except for minute points of rupture. The fact that erythrocytes can escape from a capillary is evidence that it is not permanently damaged. In the table it appears that the cases with severe hematuria have the least glomerular damage. This corresponds with clinical experience that severe hematuria without edema, hypertension or renal insufficiency affords a good prognosis. Baehr has suggested the term "benign hemorrhagic nephritis" for this form of renal disease that heals rapidly without permanent damage to the kidneys.

Our cases of the hemorrhagic type (Nos. 65 to 75) are a peculiar form of renal disease. Although hematuria was an outstanding symptom the clinical picture indicated septicemia rather than nephritis. Renal insufficiency is produced by extensive obstruction of the tubules by blood. The renal lesion resembles that found in uremia following transfusion with incompatible blood.

*Edema:* The degree of edema (subcutaneous edema, ascites, hydrothorax) is roughly indicated in the table by numerals. When the amount of edema varied during the period of observation the average condition is indicated. It will be noted that edema was absent at all times in 23 cases. Eight of the 31 uncomplicated cases of the proliferative form (Type 1a) showed no edema. There were only 28 cases in which edema was prominent (Grades 2 to 4). Edema was inconspicuous or absent in the hemorrhagic type. In three instances (Nos. 20, 21, 25) death was due, not to renal insufficiency, but to edema of the lungs and larynx. Several writers have reported cases of acute nephritis in which death was due to edema of the lungs. Murphy observed edema in 62 of 94 patients but it was never a severe complication. The presence of edema is not necessary to establish the diagnosis of any type of acute nephritis except lipoid nephrosis.

The factors that influence the development and the intensity of edema in acute glomerulonephritis are not well understood. The plasma proteins were not determined in our cases. The depletion of plasma proteins may be a factor of importance in some instances, but according to the majority of investigators they are at a normal level or only slightly reduced at the height of the edema. In 5 of our cases (hypertension and bacterial endocarditis) the severe edema was probably due chiefly to cardiac decompensation.

Some writers have described acute dilatation of the heart with

cardiac decompensation in acute glomerulonephritis (Franke, Levy). This has been noted especially in patients with marked hypertension. The cardiac dilatation was demonstrated in roentgenograms. In 3 of 27 fatal cases Murphy attributed death to heart failure, and in 15 of his cases myocardial insufficiency of varying degrees was noted. Koch reported that in 3 of 7 fatal cases of subacute glomerulonephritis death was caused by cardiac failure.

In 24 of our 31 uncomplicated cases of acute proliferative glomerulonephritis sections of liver were available for microscopic examination and 13 of these showed chronic passive congestion of some degree, as shown in Table I. Passive congestion was absent in 11, slight in 9, moderate in 2, and severe in 2 instances. There is, however, no close correlation between edema and chronic passive congestion of the liver; among 17 patients with edema only 9 had passive congestion and 2 of the 8 without passive congestion had severe edema. An increase of venous blood pressure is frequently demonstrable in acute glomerulonephritis (George Fahr).<sup>\*</sup> It appears that cardiac failure is partly responsible for edema but it does not seem to afford a complete explanation. Edema fluids in acute glomerulonephritis are usually said to have a high protein content suggesting an inflammatory origin, but Fahr and Kerkof found a low protein content when they were very careful in obtaining pure edema fluid for examination. Certainly the protein content of the edema fluid in acute glomerulonephritis is much lower than that of known inflammatory exudates.

*Blood Pressure:* The blood pressure was recorded in 47 of the 79 cases. The highest recorded blood pressure was tabulated unless it was inconsistent with other readings. The systolic pressure was below 140 mm. Hg. in 16 instances, 140 to 150 in 7, 150 to 170 in 9, 170 to 200 in 9, and over 200 in 6. Six of the cases with a blood pressure above 190 mm. Hg. were known to be instances of primary hypertension in which acute glomerulonephritis was a terminal complication. The low blood pressure in some instances was probably due to its having been recorded only a short time before death; it is known that the blood pressure often falls in the terminal stages of any disease. The patient may pass into uremia whether the blood pressure is high, low or normal. Murphy and coworkers found hypertension in 74 of 94 patients; they think that hypertension has no re-

\* Personal communication.



lation to the outcome of the disease unless it persists after other symptoms have subsided, in which case it indicates a tendency to chronicity. It is generally agreed that hypertension may not be present at all times in acute glomerulonephritis and that it may be absent entirely. In mild cases it may be present only during the first few days. It usually subsides long before albumin disappears from the urine. No case can be regarded as healed if hypertension is still present. Persistent hypertension beyond the acute stage indicates the development of chronic nephritis.

Hypertension is probably due to a diminished flow of blood through the kidneys. Bell and Pederson produced hypertension experimentally by obstructing one renal vein, and Goldblatt and co-workers produced chronic hypertension by narrowing both renal arteries. The experimental evidence supports the view that anemia of the kidney is the primary cause of the rise of blood pressure. It has been shown that hypertension occurs in Goldblatt's experiment with denervated kidneys (Page, Goldblatt *et al.*) so that it does not depend on reflexes from the anemic kidney. In acute glomerulonephritis there is a reduced flow of blood through the kidneys brought about by the widespread capillary obstruction, and it appears probable that either the anemia of the kidney or the increased resistance in the renal circulation is in some way responsible for the hypertension. The absence of hypertension in some severe cases of acute glomerulonephritis is presumably due to a failure of the heart or vasomotor system to respond to the stimulus from the diseased kidney. Volhard believes that arteriolar spasm in the kidneys precedes and causes the structural changes in the glomeruli, but the glomerular lesions develop in the absence of hypertension and they are not the type of lesion that develops elsewhere from ischemia.

*Renal Insufficiency:* In fatal cases renal function decreases rapidly during the last few days and the highest level of blood urea is observed on the last day of life. The blood urea nitrogen may be found normal or only moderately elevated 1 week before death but at a uremic level on the last day. The number of days or weeks before death when the functional test was made is indicated in the table, and it will be noted that a high blood urea nitrogen was nearly always found in the cases that were tested shortly before death. It is apparent that renal failure is usually the chief cause of death. However, the patient may develop edema of the lungs or larynx

(No. 25), or a terminal septicemia (Nos. 52 and 75), and die before the blood urea is much elevated. In the cases of acute lipoid nephrosis (Nos. 76 to 79), death was due to peritonitis in 3 instances. The associated disease was the main cause of death in several cases.

In 6 of the 7 cases with severe hematuria (Type 6) in which the blood urea nitrogen was determined it was found to be markedly elevated. These patients were suffering from bacteremia rather than from renal disease and the renal insufficiency was brought about by extensive obstruction of the tubules with blood.

Murphy found nitrogen retention in 67 of 94 cases. A patient may recover even after blood urea has reached high values. Jauré-guy and Ayala reported the recovery of a patient whose blood urea was 115 mg. per cent. Functional tests give the functional capacity of the kidneys at the time they are made but they are not a safe criterion for prognosis in acute nephritis since the renal function may rapidly improve or deteriorate.

The most reasonable explanation of impaired renal function in acute glomerulonephritis is based on the structural changes in the glomeruli. In severe cases a large proportion of the glomerular capillaries are closed more or less completely by endothelial cells and leukocytes, or by external pressure from epithelial crescents. Since the closed capillaries contain no blood they cannot take part in the formation of glomerular filtrate, and if nearly all the capillaries are closed anuria must result. However, a few open capillaries are usually found in most of the damaged glomeruli and in some nearly all the capillaries are open. The glomerular filtrate is formed from the open capillaries and presumably filtration is taking place through all open capillaries. The total glomerular filtrate is decreased in amount in severe cases and it is distributed through more tubules than under normal conditions in which only about one-third of the nephrons are active at any one time. The decrease of glomerular filtrate tends to cause retention of metabolites. The distribution of the filtrate through a larger number of tubules where it is exposed to greater reabsorption tends to produce a concentrated urine. With greater tubular reabsorption it is probable that more urea passes back into the blood. A similar explanation of abnormal renal function has been offered by Dunn and by Fremont-Smith and his associates.

Protein must escape through open glomerular capillaries and

heavy proteinuria does not necessarily indicate irreparable injury. The amount of protein in the urine decreases in contracted kidneys since there is a great reduction in the number of open capillaries.

The erythrocytes escape from minute ruptures of open capillaries and hematuria would therefore have no serious significance except for the obstructive lesions that may be associated with it.

Another factor that should be given consideration in determining the anatomical basis of disturbed renal function is the swelling of the kidneys. The kidneys are nearly always much larger than normal and their capsules are tense. The swelling is not due to an increased amount of blood, since the tissue is pale and microscopically the blood content seems less than normal. However, there is some enlargement of the tubules from swelling of their cells or from dilatation of their lumens which tends to compress the intertubular capillaries. Occasionally there is some interstitial edema which also tends to compress capillaries and reduce the rate of blood flow.

*Relation to Infection:* Our observations agree with those of nearly all observers that there is usually a history of infection preceding acute glomerulonephritis. By far the most common antecedent infections are those of the upper respiratory tract, *i.e.* sore throat, common cold, bronchitis, scarlet fever, and so on. A large variety of infectious processes appear occasionally immediately preceding the nephritis, *i.e.* smallpox, chickenpox, infected wound, impetigo contagiosa, and so on. In most instances the antecedent infection produces a lesion of mucous membrane or skin which allows the entrance of bacteria, especially streptococci into the tissues. Proliferative glomerulonephritis is usually caused by streptococci, less frequently by pneumococci or other organisms. The difficulty in establishing the exact etiological agent is due to the uniform absence of bacteria from the glomerular lesion. It appears that the diffuse glomerular lesions are produced by a soluble toxic substance and that if the bacterial bodies lodge in the glomeruli an embolic type of lesion results.

The interval between scarlet fever and the onset of nephritis is usually 2 to 3 weeks and this fact has suggested to several investigators that the glomerular lesion does not develop until the individual has become hypersensitive to the bacterial protein. Fahr and Masugi have sought to establish this principle by demonstrating that a severe glomerulonephritis results from injection of foreign

serum into the renal artery of an animal previously sensitized to the corresponding serum. A typical glomerulonephritis may indeed be produced in this way but it seems more comparable to the Arthus phenomenon than to glomerulonephritis.

In many instances the interval between the initial infection and the onset of symptoms of nephritis is too short for sensitization to have developed.

## II. VASCULAR LESIONS

Acute renal insufficiency may be produced by thrombosis of the renal arteries or by widespread thrombosis of small arteries with formation of multiple infarcts.

### (A) *Thrombosis of Renal Artery*

The patient (35-1943), a female, 71 years of age, had had a right nephrectomy for calculus 30 years previously. She developed extreme oliguria and died of uremia. The left renal artery was almost occluded by an old thrombus and there were numerous small infarcts throughout the kidney.

### (B) *Widespread Thrombosis of Small Arteries*

Male (35-730), 9 years old. During the course of an empyema following pneumonia the patient developed anuria and died of uremia. The systolic blood pressure was 120 mm. Hg. The blood urea nitrogen was 182 mg. per cent on the day before death. There was no edema. The kidneys weighed 350 gm. and were filled with small infarcts caused by thrombosis of small arteries. The greater part of the cortex of both kidneys was necrotic. Cortical necrosis of the kidneys has been reported frequently in eclampsia and a similar lesion has been described in males associated with infectious processes.

### (C) *Polyarteritis Nodosa*

Male (32-588), 64 years old. The duration of the illness was about 3 weeks. There was no albuminuria or edema. The urinary output was not noted. The renal disease was not recognized clinically but the extensive infarction of the kidneys found at postmortem is sufficient to establish the diagnosis of renal insufficiency. The kidneys weighed 400 gm. The infarcts were related to lesions of the

small arteries. It is well known that patients suffering from polyarteritis nodosa may develop uremia from extensive lesions of the small renal arteries.

### III. INTERSTITIAL LESIONS

#### (A) *Acute Interstitial Nephritis*

A male (25-1059), 23 years of age, developed sore throat, fever and general malaise on December 12th. On December 19th he was admitted to the hospital with a typical scarlet fever rash and sore throat. At this time the urine was negative. He improved for a few days and then became very toxic on December 24th. The blood pressure was 80/42. His condition gradually became worse and death occurred on December 30th. The urine showed only a trace of albumin until December 29th, when there was a heavy albuminuria with many casts and leukocytes. There is no record as to the diuresis. On December 30th the blood urea nitrogen was 174.3 mg. per cent and creatinine 10.6 mg. per cent.

The kidneys were enormously enlarged, the right weighing 520 gm., the left 570 gm. Microscopically there was a massive infiltration of the interstitial tissues with mononuclear and polymorphonuclear leukocytes. The renal insufficiency was probably due to compression of the blood vessels and tubules throughout the kidneys by the massive interstitial exudate.

#### (B) *Acute Lymphatic Leukemia*

The patient (33-1069), a female, 40 years of age, was well until June 1, 1933, when she first noticed purpura and discoloration following slight bruises. On June 12th there was a severe hemorrhage following extraction of a tooth. She was admitted to the hospital June 17th, complaining of weakness and petechial hemorrhages over the greater part of the body. The blood picture was that of lymphatic leukemia. The urine showed heavy albuminuria and many erythrocytes. There was no edema. June 17th the blood urea nitrogen was 105.4 mg. per cent and the creatinine 7.5 mg. per cent. Death occurred June 20th, 1933. The kidneys weighed 370 gm. and showed widespread leukemic infiltration of the cortices. The effect on the kidney is similar to that of acute interstitial nephritis.

## IV. TUBULAR DISEASES OF THE KIDNEYS

(A) *Pure Tubular Degeneration*

(a) *Mercuric Chloride Nephritis*: There are 7 cases of mercuric chloride poisoning in our records and in 6 of these death was due to renal insufficiency.

1. A male, 51 years of age, who was suffering from carcinoma of the colon, took an unknown amount of mercuric chloride and died 30 minutes later. At postmortem the kidneys showed no gross or microscopic alterations. The kidneys are presumably injured but some hours must elapse before any structural changes can be detected.

2. A male, 81 years of age, was admitted to the hospital 2 days before his death. He was uncertain as to when he took the poison. No urine was voided but 50 cc. was removed by catheter. This contained only a trace of albumin. There was no edema. The blood urea was 218 mg. per cent. At postmortem no colitis was found. The kidneys weighed 334 gm. Many cells in the convoluted tubules are necrotic and desquamated, but nearly all the tubules are lined with fairly thick, deeply stained cells. The lumens are not dilated.

3. A female, 45 years of age, died 7 days after taking an unknown amount of mercuric chloride. Very little urine was passed during this period. There was no edema. At postmortem ulcerative colitis and ileitis was found. The kidneys weighed 350 gm. The convoluted tubules are not distended but their lumens are filled with necrotic and desquamated cells. Numerous partly formed epithelial casts are noted. There is no evidence of tubular regeneration; there are no dark flattened cells lining the tubules.

4. A female, 29 years of age, died 10 days after inserting a 5 gr. tablet of mercuric chloride into her vagina. She complained of a severe burning pain, chills, fever and headache. The blood pressure was 110/70 on the 2nd day, and 98/55 on the 3rd day. Ulceration of the gums developed on the 7th day. No urine was voided except a small quantity on the 6th and 7th days, which contained some albumin and casts. On the 7th day there was edema of the right optic disc and a slight subcutaneous edema. At postmortem gangrenous cystitis, ulcerative colitis and acute pericarditis were found. The kidneys were enormously enlarged, weighing 825 gm. The con-

volute tubules are all enormously dilated and lined by very thin epithelial cells that contain no granules. In some tubules the lining cells are completely absent, but no necrotic cells are to be seen (Fig. 7). The few casts that are present seem entirely inadequate to explain the tubular distention.

5. A female, 24 years old, died 11 days after taking 10 gr. of mercuric chloride. Some of the poison was removed by vomiting and gastric lavage. There was no record of the diuresis. The urine contained a small amount of albumin. At postmortem a non-ulcerative colitis was found. The kidneys weighed 370 gm. There are a few necrotic tubules. There is not much tubular dilatation and the tubules are lined with fairly thick dark cells.

6. A female, 14 years of age, took 22.5 gr. of mercuric chloride. She vomited shortly afterwards. Oliguria developed on the 2nd day and persisted in a severe form until the 10th day, but there was some increase of the urinary output during the last 3 days. The gums became sore on the 3rd day. On the 13th day the blood pressure was 104/50. The blood urea nitrogen was 74.6 mg. per cent on the 4th day, and 124 mg. per cent on the 10th day. There was no edema. Death occurred on the 13th day. The kidneys weighed 370 gm. There is a heavy lymphocytic infiltration in the medullae. The tubules are markedly dilated and lined by thin dark epithelium. No necrotic cells are visible and there are only a few casts.

7. A female, 26 years of age, died 16 days after taking 25 gr. of corrosive sublimate. She complained of severe abdominal pain and became semicomatose on the 2nd day. A severe stomatitis developed. The systolic blood pressure was 126 mm. Hg., on the 2nd day, 164 mm. Hg. on the 10th day, and 128 mm. Hg. on the 16th day. The blood urea nitrogen was 20.5 mg. per cent on the 2nd day, 140 mg. per cent on the 9th day, and 162 mg. per cent on the 15th day. There was a heavy albuminuria at first but later only a trace was present. There was severe oliguria at first but during the latter part of the illness the urinary output was about 1000 cc. daily. The specific gravity of the urine was never above 1014. There was no edema. The hemoglobin decreased from 90 to 65 per cent.

At postmortem a few small areas of ulcerative colitis were found. The kidneys weighed 370 gm. The convoluted tubules are all dilated and lined by low dark epithelial cells (Fig. 8). A few necrotic

tubules are noted. There are numerous casts in the collecting tubules. There is a definite interstitial edema and an occasional glomerular abscess is noted.

The most prominent symptom in corrosive sublimate poisoning is oliguria which usually progresses to anuria. As a result of the decreased diuresis there is a progressive increase of urea and other blood metabolites and death results from uremia. Occasionally, even in cases that terminate in uremia, there is an increased diuresis after several days (Case No. 7) and the urinary output may approach the normal. In such instances recovery may take place (Mach and Oppikofer) or the blood urea may continue to increase as in our Case 6. It will be noted in our case that the kidneys were unable to concentrate the urine above 1014. With such a loss of concentrating power a diuresis of 1000 cc. daily is insufficient to prevent the increased accumulation of blood metabolites.

There is usually a large amount of albumin in the urine during the first day or two, but later there is very little. This indicates that there is a primary glomerular injury which soon subsides.

Edema is usually absent entirely or very slight in amount, but when large quantities of fluid are given it may become very marked (Sollmann and Schreiber).

The blood pressure is sometimes moderately increased during the stage of severe oliguria. The blood urea increases steadily in the cases that terminate fatally. In the case reported by Mach and Oppikofer the blood urea reached 644 mg. per cent on the 11th day, but the patient recovered. The high level of the blood metabolites is due in large measure to renal failure, but the depletion of the body fluids by vomiting and diarrhea is a contributory factor. Roth found a striking decrease of blood chloride in his patient and attributed the uremia to hypochloremia.

In persons who die within a few hours after taking the poison no renal changes are demonstrable. In those who die after several days there is usually found a marked necrosis and desquamation of the cells of the convoluted tubules. Not all the cells in the tubules become necrotic; many survive. Often it appears that the inner half or two-thirds of the cell becomes necrotic and desquamates while the basal portion containing the nucleus persists. Regeneration probably takes place from the basal portion of the cell.

In those who die 10 or more days after ingestion of the poison the



kidney presents quite a different microscopic appearance. Very little necrosis is now seen. The tubules are lined with cells that appear much darker than the normal cells in hematoxylin-eosin preparations. The mitochondrial granules are not to be seen. The cells are usually flattened or cubical in shape (Fig. 8) and the lumens of the tubules are usually widened. The dark cells are interpreted as new epithelium formed by regeneration. The decreased height of the cells is partly responsible for the wide lumen, but there are numerous casts in the collecting tubules which may obstruct the outflow of urine. There is often some interstitial edema.

The glomeruli show no structural changes. Evidently there is a moderate initial glomerular injury since there is heavy albuminuria during the first day or two, but the disappearance of albumin later indicates only temporary injury. In frogs poisoned with mercuric chloride Richards found the glomerular circulation normal although no urine reached the renal pelvis. By injections with Janus green Moore and Hellman concluded that there is no decrease in the number of open glomeruli in the anuric stage of mercuric chloride nephritis.

Richards demonstrated in the frog's kidney that in mercuric chloride poisoning the glomerular filtrate is formed in normal amount and composition. It evidently diffuses back into the interstitial tissue through the denuded tubular wall or through cells that have no power of selective retention. Phenol red diffuses back into the tissue when injected into the tubule. One must suppose that the regenerated cells seen in the human renal tubules in the later stages of the disease have not yet attained a normal functional capacity.

It is clear that mercuric chloride nephritis is a purely tubular disease and that the characteristic disturbance in tubular disease is oliguria or anuria and not albuminuria or edema. The conception of lipoid nephrosis as tubular disease finds no support in the study of the mercuric chloride kidney.

#### *(b) Other Types of Pure Tubular Degeneration*

8. 33-1730. A male, 56 years of age, had had intermittent nausea and diarrhea for 4 years prior to admission. He died 11 hours after he entered the hospital. The blood pressure was 82/50, and no urine could be obtained by catheter. The blood urea nitrogen was 116 mg. per cent, and the van Slyke 26 volumes per cent. He was consider-

ably emaciated. At postmortem 300 cc. of urine was obtained from the bladder and this showed a specific gravity of 1010, albumin + and a few erythrocytes. There was a terminal bronchopneumonia. The kidneys weighed respectively 160 gm. and 180 gm. Microscopically there is found a severe hydropic degeneration of all the convoluted tubules. There is no glomerulitis and the tubular lesions are severe and amply sufficient to explain the uremia. The duration of the renal disease cannot be determined from the meager clinical history.

9. 35-483. An obese white female, 24 years of age, was admitted to the hospital on March 17th. For 10 days prior to admission she had been confined to bed with chills and fever. She had vomited occasionally and had a marked oliguria. On admission she was semicomatose; the blood pressure was 130/80, and the temperature 99.2° F. The urine was always scanty in amount and contained a large amount of albumin. The hemoglobin was 87 per cent, and there were 16,500 leukocytes per cmm. On March 19th the blood urea nitrogen was 109 mg. per cent, and creatinine 1.8 mg. per cent. On March 22nd the blood urea nitrogen was 123 mg. per cent, and the plasma chlorides 472 mg. per cent. Death occurred on March 23rd.

At postmortem there was found a purulent pericarditis and a moderate bronchopneumonia, both of which appeared to be terminal secondary infections. The kidneys weighed 290 gm. each and were very pale. Microscopically most of the convoluted tubules show extreme hydropic degeneration (Fig. 9) and the remainder are greatly dilated and lined by a thin epithelial layer. This is a pure degenerative tubular lesion. There is no glomerulitis. There is no resemblance to the mercuric chloride kidney, and careful questioning of the relatives of the patient failed to bring out anything suggestive of any type of chemical poisoning.

#### *(B) Obstructive Tubular Disease from Blood Transfusion*

10. 31-1823. A male, 51 years of age, was admitted to the hospital October 7th, with an inoperable carcinoma of the stomach. His hemoglobin was 34 per cent and his red cell count 1,740,000. On October 19th a blood transfusion was begun. After 100 cc. had been given the transfusion was discontinued because the patient went into shock. It was later determined that the donor belonged to Group II

and not to Group IV as did the recipient. The patient recovered from shock but developed an oliguria which persisted. In spite of a large fluid intake the urinary output was 200 cc. on October 20th, no urine was obtained on October 23rd, 400 cc. on October 25th, 575 cc. on October 29th, but very little urine after this date. On October 29th, no phenolsulphonephthalein was excreted in 2 hours. The non-protein nitrogen was 151 mg. per cent. Death occurred November 2nd.

The kidneys weighed 250 gm. each. On section the surfaces were pale. Microscopically a majority of the collecting tubules are plugged with hemoglobin casts and there is a moderate distention of all the convoluted tubules. There are no other changes.

11. 34-1183. A female, 29 years of age, was admitted to the hospital June 12th. She had induced an abortion about 2 weeks previously and now had puerperal septicemia. The hemoglobin was 40 per cent and the leukocyte count was 9000. There was continuous fever. The blood pressure was 120/80. The urine showed only a faint trace of albumin. On June 19th a blood transfusion was given, but before the transfusion was completed the patient developed a severe shock. She had been given 360 cc. of citrated blood from her husband. The preliminary tests indicated that both donor and recipient belonged to Group I. She recovered from the shock but during the next 24 hours passed only 50 cc. of urine. The urine showed a specific gravity of 1035, a 4 plus albumin and a large amount of hemoglobin. Although hemoglobin soon disappeared from the urine a marked oliguria persisted. In spite of a large fluid intake and the use of spinal anesthesia the output of urine varied from one-fourth to one-tenth of the fluid intake. A heavy albuminuria persisted after disappearance of the hemoglobin, and there were many erythrocytes in the urine. The hemoglobin remained around 30 per cent and the erythrocyte count about 2,000,000. On June 20th the icterus index was 30 and a quantitative determination of bilirubin gave 19 parts per million. The blood urea nitrogen was 52 mg. per cent on June 20th, 90 on June 29th, 115 on July 3rd, and 144 on July 5th. During this time blood creatinine rose from 3.5 mg. per cent to 9.9. On July 9th dyspnea developed which was found to be due to edema of the lungs. The patient did not become drowsy but remained awake and alert until death on July 9th.

At postmortem there was found marked edema of the lungs and

500 cc. of clear fluid in the peritoneal cavity. The kidneys were enlarged, weighing 215 gm. and 240 gm. respectively. The cortices were gray in color. Microscopic examination shows a rather marked dilatation of all the renal tubules and in some parts of the cortex there is interstitial edema (Fig. 10). There is no glomerulitis and none of the tubules is necrotic. A large number of the collecting tubules and loops of Henle are obstructed by hemoglobin casts (Fig. 11).

12. 36-224. A female, 27 years of age, underwent a Cesarean section because of placenta previa. Following the operation five blood transfusions were given, each about 600 cc. The first two transfusions were given 12 hours and 18 hours respectively after the operation; the other three were given at 24 hour intervals. There are no details available as to the reaction after the transfusions. The patient developed a severe oliguria, about 60 cc. of urine daily, and died 1 week after the operation. The blood urea nitrogen rose to 120 mg. per cent. Only sections of the kidneys were available for study. The microscopic appearances are the same as in the two previous transfusion kidneys; there is a marked dilatation of all the tubules with some interstitial edema, and the collecting tubules are plugged with hemoglobin casts.

It has been known for many years that hemoglobinuria may follow blood transfusions. After the recognition of the blood groups it was determined that hemolysis and hemoglobinuria rarely occur unless the patient is transfused with blood from a donor of a different blood group. Ponfick (1875) transfused various animals with blood from other species and frequently obtained hemoglobinuria, anuria and death of the animals. He observed casts of hemoglobin in the tubules. Lemke (1925) reported a patient who died 6 days after transfusion; there were oliguria, hemoglobinuria and clinical signs of uremia. He noted some injury of the tubular epithelium and hemoglobin masses in the tubules.

The most frequent cause of death in blackwater fever is anuria and uremia, and this has been attributed by several investigators to plugging of the renal tubules with hemoglobin casts. The kidneys in such cases of blackwater fever resemble the transfusion kidneys rather closely. Yorke and Nauss (1911) were able to produce suppression of urine and uremia in rabbits by intravenous injections of hemoglobin. The renal tubules, especially the collecting tubules, were plugged with casts largely composed of hemoglobin. The kid-

neys of the experimental animals correspond entirely with those from blackwater fever and those resulting from transfusion of incompatible blood. It is important to note that suppression of urine in the rabbits was obtained only when they were on a dry diet; it did not result when they had free access to water. The authors believed that the dry diet caused concentration of the urine which resulted in precipitation of hemoglobin. Baker and Dodds (1925) studied the kidneys of two persons who died following transfusion with incompatible blood. They attributed the uremia to obstruction of the tubules by casts of hemoglobin. They confirmed the experimental observations of Yorke and Nauss, but noted that the urine from the rabbits with uremia was of a dark brown color and highly acid, while the rabbits in which no urinary suppression developed had a reddish alkaline urine. By feeding rabbits on a diet of oats and bread, without any green food, they kept the urine permanently acid although the animals were allowed unlimited water to drink. Injections of hemoglobin into rabbits with an acid urine caused urinary suppression. Hemoglobin is precipitated from acid urine.

Lindau (1928) gave a good discussion of the clinical symptoms and reported the postmortem findings in 3 cases. He found moderate injury of the convoluted tubules in 2 cases and severe degeneration in 1. The tubules were not dilated. He noted casts in the tubules but considered degeneration and necrosis of the tubular epithelium the chief cause of the anuria.

Halter (1930) described the kidneys of a patient who died of uremia on the 10th day after a blood transfusion. He noted that the tubules were dilated and that many of them, especially those in the medulla, were plugged with hemoglobin and other forms of casts. He attributed uremia to mechanical obstruction of the tubules. Irsigler, 1932, agreed with Halter that mechanical obstruction of the tubules by casts is the cause of tubular dilatation and uremia. He noted also a little lymphocytic infiltration of the interstitial tissue.

Bordley (1931) reported three severe transfusion reactions, one of which resulted fatally. The kidneys in this instance weighed together 589 gm. The tubules were dilated and there was edema and lymphocytic infiltration of the interstitial tissue. There were many necrotic epithelial cells. Casts were evidently not considered an important cause of the renal insufficiency.

Hesse and Filatov (1932, 1933) proposed the theory that uremia is due to spasm of the renal arteries. They performed experiments on dogs which showed a decrease of kidney volume following transfusion unless the kidneys were previously denervated. They recommended, as therapeutic procedures in uremia following transfusion, denervation of the kidneys and transfusion with compatible blood to release the vascular spasm.

It is to be noted in connection with this hypothesis that spinal anesthesia in one of our cases (No. 11) had no influence on the oliguria. Johnson and Conway had previously reported that spinal anesthesia was without effect.

Johnson and Conway (1933) described the kidneys of a patient who died of uremia on the 18th day after a transfusion. Dr. F. B. Mallory found areas of focal necrosis in the liver and adrenals; the kidneys showed severe damage of the tubular epithelium with evidences of regeneration.

DeGowin and Baldrige (1934) described and illustrated the changes in the kidneys in a patient who died on the 10th day following transfusion. The collecting tubules of the medulla were filled with hemoglobin casts; the convoluted tubules were moderately dilated and showed a low cuboidal epithelium resembling the late stage of mercuric chloride poisoning. There was a notable interstitial edema in the cortex.

Terplan and Javert (1936) described the kidneys from a case of hemoglobinuria with fatal uremia following excessive administration of quinine in early pregnancy. The collecting tubules were obstructed by hemoglobin casts as in the transfusion kidneys.

In my 11 cases of hemorrhagic nephritis the tubules were obstructed for the most part by compact masses of erythrocytes but in 1 case many of the casts were composed largely of hemoglobin.

It appears that suppression of urine and uremia following transfusion with incompatible blood is due in large measure at least to mechanical obstruction of the tubules by casts of hemoglobin. The explanation offered by Baker and Dodds that the acidity of the urine is the determining factor in causing the precipitation of hemoglobin in the renal tubules seems well established by their experiments. In the light of their work alkalization of the urine preliminary to transfusion might be worth while in case there is any uncertainty as to the compatibility of the blood to be used.

## V. EXTRARENAL UREMIA

Extrarenal uremia refers to uremia that occurs as a result of extrarenal influences when the kidneys are structurally normal. This form of uremia is not at all uncommon and will be found frequently if the blood urea nitrogen is determined during the last days of life or immediately after death. Frequently clinical evidences of uremia are present. In many instances of extrarenal uremia the kidneys are found to be entirely normal or only slightly altered; in other cases there are renal changes such as enlargement and dilated tubules but the structural changes are inadequate to explain renal insufficiency.

*(A) With Some Tubular Injury*

13. 33-2051. A female, 39 years of age, underwent a vaginal hysterectomy on November 21st. Her temperature rose on the 8th postoperative day and continued to be elevated thereafter. On November 28th the urine contained a large amount of albumin and many pus cells and erythrocytes. Several subsequent examinations of the urine gave similar findings. There was no record of the quantity of urine. On December 12th only a trace of phenolsulphonephthalein was excreted in 2 hours. Death occurred on December 13th. Blood taken 4 hours after death showed urea nitrogen 113 mg. per cent, and creatinine 4 mg. per cent.

At postmortem a pelvic abscess was found and there was a moderate amount of bronchopneumonia. The kidneys weighed 110 gm. and 210 gm. respectively. Microscopically there is a widespread severe hyaline granular degeneration of the convoluted tubules but no necrosis. There is no glomerulitis. In the medulla there are some casts and some foci of lymphocytes. In this case there is evidence that tubular injury is partly responsible for the renal insufficiency.

14. 36-235. A male, 73 years of age, sustained a severe injury of his right leg on January 20th, which was followed by severe infection of the entire leg below the knee. He was admitted to the hospital on January 30th and the infection was treated by hot packs and incisions. His temperature gradually rose to 107.8° F. and he died on February 4th with evidence of septicemia. The urine showed a little albumin and many casts (no note on the diuresis). The hemoglobin was 73 per cent and the leukocyte count 31,500 (93 per cent poly-

morphonuclears). The blood urea nitrogen was 169 mg. per cent on February 2nd.

Postmortem examination revealed severe cellulitis of the leg, a severe portal cirrhosis of the liver and terminal bronchopneumonia. The kidneys weighed 170 gm. and 190 gm. each and their cortices were cloudy. Microscopically the cells of the convoluted tubules are all swollen and vacuolated but there is no necrosis and no dilatation (Fig. 12). There is no glomerulitis. The tubular injury is not sufficient to explain the uremia and we must therefore suppose that some extrarenal factor is partly responsible.

15. 36-468. A male, 34 years of age, had taken an unknown amount of phenobarbital. He fell and sustained a wound of the scalp in the occipital region. He was admitted to the hospital immediately afterwards in coma. It was not known whether the coma was caused by the fall or by the drug, but the postmortem revealed no fracture of the skull or bruising of the brain. On admission January 31st he was in extreme shock; the blood pressure was 60/0. The patient slowly recovered from shock but remained in a stuporous condition until his death on February 9th. The urinary output was 900 cc. on January 31st, but thereafter the daily diuresis varied from 60 cc. to 190 cc. The total daily fluid intake varied from 2500 cc. to 3890 cc. He vomited occasionally. On February 5th a generalized anasarca developed. The blood urea nitrogen was 38.9 mg. on February 2nd and 63 mg. per cent on February 4th; creatinine on corresponding dates was 3.8 mg. per cent and 5 mg. per cent. The blood pressure on February 5th was 170/86. The right kidney was decapsulated on February 7th, but there was no improvement in the diuresis. Death occurred on February 9th in clinical uremia. The urine showed a large amount of albumin after February 2nd.

The kidneys weighed 210 gm. and 205 gm. each. Microscopically about half of the convoluted tubules are markedly dilated and lined by thin flattened epithelium, but the others appear fairly normal. There are many casts in the collecting tubules which are probably sufficient to explain the dilated cortical tubules. There is no glomerulitis and there is no necrosis. The uremia is largely due to extrarenal factors but tubular obstruction is a contributory factor.

16. 34-1449. A female, 35 years of age, became ill on August 8th with nausea, severe vomiting and epigastric pain. On August 13th jaundice appeared. On admission, August 15th, the temperature



was normal and the blood pressure 140/86. There was a slight jaundice. The specific gravity of the urine was 1026 and there was a large amount of albumin. There was a persistent oliguria (about 400 cc. daily) and subsequent urine examinations showed a specific gravity from 1011 to 1015, and moderate to severe albuminuria. The blood urea nitrogen on August 22nd was 175 mg. per cent and creatinine 8.8 mg. per cent. Death occurred on August 23rd.

At postmortem there was a slight jaundice but no edema. The liver showed areas of necrosis and moderate fatty metamorphosis. There were multiple small abscesses in the pancreas. The kidneys were greatly enlarged, weighing 290 gm. and 300 gm. each. Microscopically the cortical tubules are all moderately dilated and most of the collecting tubules and loops of Henle are obstructed by casts. There is some lymphocytic infiltration in the medullae. There is no glomerulitis. The obstruction of the tubules by casts is a definite contributory cause of the uremia.

17. 34-919. A male, 51 years of age, was admitted to the hospital on May 19th. For several years he had drunk large quantities of alcoholic liquors, often as much as one quart daily. For 5 days preceding admission he had been unable to eat, had been vomiting steadily and had passed no urine for the last 3 days. The blood pressure was 150/84. The sclerae showed a slight jaundice. The vomiting persisted. He passed no urine and only 30 cc. was obtained by catheter during the 3 days he was hospitalized. He was given large amounts of fluid by hypodermoclysis and intravenously. The urine showed a trace of albumin. The icteric index was 156 on May 20th. On May 21st the blood urea nitrogen was 140 mg. per cent and creatinine 10 mg. per cent. The hemoglobin was 84 per cent and the red cell count 4,820,000. The temperature remained normal. The patient died in coma on May 22nd.

At postmortem the body was obese, the liver weighed 2065 gm. and was very fatty, and there was terminal bronchopneumonia. The kidneys weighed 277 gm. and 288 gm. each; their cortices were thick and cloudy. Microscopically there are no renal changes except some swelling and vacuolization of the cells of the convoluted tubules. This is evidently chiefly an extrarenal uremia from loss of fluid but the marked swelling of the kidneys indicates some renal injury.

18. 34-1059. A male, 36 years of age, was admitted to the hospital on June 5th. His illness began 10 days previously with vomiting.

He could retain no solid food and only a small amount of liquid. This condition continued until his death on June 17th. He was given fluids subcutaneously and intravenously but continued to vomit. The specific gravity of the urine ranged from 1010 to 1018, and the amount of albumin varied from 1 to 4 plus. There was no record of the amount of diuresis but he did not have anuria. There was no excretion of phenolsulphonephthalein in 2 hours. His blood pressure was continuously about 180/110. The hemoglobin was 76 per cent, and the leukocyte count rose from 9500 to 19,000. On June 13th the blood urea nitrogen was 189 mg. per cent and creatinine 8.3 mg. per cent. Death occurred on June 17th.

At postmortem the only notable changes were in the kidneys which weighed 250 gm. each. Microscopically there is no glomerulitis, the tubules are all moderately dilated but there are only a few casts and there is no necrosis. The tubule cells are not deeply stained, as they are in late bichloride poisoning.

This appears to be largely extrarenal uremia from loss of fluids but the kidneys are not normal.

19. 34-1901. A white female, 30 years of age, was admitted to the hospital on November 1st in diabetic coma. She was known to have had diabetes for several months but had received no treatment. Coma developed on the afternoon of October 31st. She had been in coma about 10 hours when admitted. The blood pressure was 70/40 and the breathing was of Kussmaul type. The urine showed specific gravity 1020, albumin 2 plus, sugar 4 plus, acetone and diacetic acid. The blood sugar was 625 mg. per cent and the van Slyke 8 volumes per cent. Under treatment the blood pressure soon rose to 110/60, blood and urine sugar were decreased, and she slowly regained consciousness. There was oliguria at first but this improved somewhat by the 3rd day. The blood urea nitrogen was 41 mg. per cent on November 1st, 56 on November 2nd, and 68 on November 3rd. On November 2nd the phenolsulphonephthalein output in 2 hours was only 2 per cent. Death occurred on November 5th.

The kidneys were enormously enlarged, weighing 280 gm. and 320 gm. respectively. Microscopically the only notable change is a uniform, rather marked tubular dilatation (Fig. 13). There are only a few casts. The interpretation is extrarenal uremia with contributory renal injury.

20. 30-1937. A male, 18 years of age, was admitted to the hospital

December 21st. He had had rather severe diabetes for  $2\frac{1}{2}$  years, and had had several attacks of coma. He had been under insulin treatment but had been careless in his self management. On the day of admission he had become weak, sleepy and tired, and had begun to vomit. He developed dyspnea and orthopnea. The urine showed albumin 4 plus, many casts, sugar 4 plus, acetone and diacetic acid. The blood sugar was 530 mg. per cent and the van Slyke 14 volumes per cent. The red cell count and hemoglobin were normal and the leukocyte count 13,600. On December 22nd, after insulin, the urine was sugar-free, the blood sugar 60 mg. per cent, the blood urea nitrogen 17 mg. per cent, and the van Slyke 25 volumes per cent. The phenolsulphonephthalein excretion on this day was 58 per cent in 2 hours. On December 23rd the urine showed sugar 1 plus, albumin 3 plus, many casts and acetone, and the blood sugar was 350 mg. per cent. On December 24th the patient developed anuria, the blood urea nitrogen was 67 mg. per cent, and the van Slyke 9 volumes per cent. Very little urine was voided after December 23rd. On December 25th the blood pressure was 140/66. Death occurred on December 25th.

Blood taken after death showed urea nitrogen 123 mg. per cent and creatinine 5 mg. per cent. There was no subcutaneous edema, but the peritoneal cavity contained 300 cc. and the pleural cavities 600 cc. of clear fluid. There was terminal bronchopneumonia. The kidneys were greatly enlarged and very pale, weighing 240 gm. and 220 gm. each. Microscopically all the tubules are enormously dilated and lined by thin, eosin-staining cells (Fig. 14). There is no glomerulitis and there are only a few casts. The diagnosis is extrarenal uremia with renal injury.

### (B) *Without Renal Injury*

In this group of cases there is marked renal insufficiency but the kidneys are practically normal on both macroscopic and histological examination.

21. 34-194. *Diabetic coma.* A negress, 63 years of age, was admitted to the hospital in coma on January 28th. On the day of admission she had had several convulsions, during which she became unconscious. She had been unconscious 3 hours prior to admission. The blood pressure was 152/90. The hemoglobin and red cell count were normal, and the leukocyte count was 31,200. The patient was

incontinent and no urine was obtained for examination. Death occurred on the morning of January 31st.

A postmortem sample of blood showed blood sugar 600 mg. per cent, urea nitrogen 89.6 mg. per cent and creatinine 4.2 mg. per cent. Postmortem examination revealed an extensive bronchopneumonia and a small carcinoma of the head of the pancreas. The kidneys weighed 100 gm. and 140 gm. each. There were cortical scars from atherosclerosis. Microscopically the kidneys are normal.

22. 34-219. *Gangrenous pancreatitis*. A male, 68 years of age, developed a sudden attack of abdominal pain on the morning of January 20th. He vomited twice that afternoon. He was admitted to the hospital on the afternoon of January 20th, at which time his entire abdomen was distended and tympanitic. There was evidence of paralytic ileus. He was given fluids intravenously and nasal suction. His general condition seemed to improve but he continued to vomit occasionally. Five specimens of urine were examined, two of which showed a trace of albumin while the others were normal. The leukocyte count ranged from 14,650 to 19,100. On January 27th the blood urea nitrogen was 86.8 mg. per cent and creatinine 2.5 mg. per cent. Death occurred January 28th.

The principal findings at postmortem were gangrenous pancreatitis and cholelithiasis. The kidneys were enlarged, weighing 219 gm. and 242 gm., but on microscopic examination they appear entirely normal.

23. 36-1055. *Gangrenous pancreatitis*. A male, 45 years of age, developed an attack of acute abdominal pain on May 22nd and was brought to the hospital. There was nausea but no vomiting. On the same day the gall-bladder and appendix were removed; the gall-bladder showed an inactive old cholecystitis and was filled with stones. He was given intravenous glucose and nasal suction. He perspired profusely almost constantly and vomited occasionally. A specimen of urine on May 25th showed albumin 3 plus, and granular casts, and the blood urea nitrogen was 53 mg. per cent. There is no record of the diuresis. The patient gradually became stuporous and irrational. On May 29th the blood urea nitrogen was 142 mg. per cent and creatinine 8.8 mg. per cent. Muscular twitchings all over the body were noted on May 30th and May 31st. Death occurred June 1st. Postmortem examination revealed acute gangrenous pancreatitis and generalized peritonitis. The kidneys weighed 150 gm.

and 175 gm. and were normal on gross and microscopic examination.

24. 34-481. *Paralytic ileus*. A young male, 19 years of age, sustained a fracture of the second lumbar vertebra on February 20th. He was in good condition until March 6th when he began to vomit and ceased to have bowel movements. On March 10th his abdomen was greatly distended. The urine on this day showed a specific gravity of 1020 and no albumin. He vomited continuously after March 6th. The blood pressure remained about 106/70. He was given large amounts of intravenous saline solution. Death occurred March 14. A postmortem sample of blood showed urea nitrogen 134 mg. per cent; creatinine 6.85 mg.; non-protein nitrogen 313 mg.; and total blood chloride 460 mg. Postmortem examination revealed a paralytic ileus. The kidneys weighed 170 gm. and 200 gm., and show no gross or microscopic evidence of disease.

25. 34-548. *Primary peritonitis*. A male, 62 years of age, developed primary peritonitis and died 8 days after the onset of symptoms. He vomited continuously from the onset of his illness. He was under observation only during the last day of his life. A small amount of urine was obtained by catheter. The specific gravity of the urine was 1020, albumin 2 plus, and many granular casts were present. The blood urea nitrogen was 109 mg. per cent. The blood pressure was 100/60.

On postmortem examination the peritoneal cavity contained 1500 cc. of purulent fluid. The kidneys weighed 140 gm. and 130 gm. Microscopically the kidneys are practically normal.

26. 34-929. *Streptococcic infection*. A male, 71 years of age, was admitted to the hospital on May 19th. On May 14th he had noted an infection of the right ankle, which spread over his entire leg during the next 2 days. The right inguinal nodes became enlarged and tender. On admission his temperature was 101.6° F. and his blood pressure 116/40. The urine showed a specific gravity of 1009 and a trace of albumin. On May 24th the urine showed a specific gravity of 1017 and albumin 1 plus; the leukocyte count was 37,000; the blood urea nitrogen was 156 mg. per cent and creatinine 5 mg. Death occurred May 24th.

On postmortem examination a severe hemolytic streptococcic infection of the leg was noted. The kidneys weighed 170 gm. and 200 gm. and their cortices were pale. Microscopically the kidneys are normal.

27. 35-2028. *Suppurative cholangitis*. A female, 51 years of age, was admitted to the hospital December 13th, complaining of upper abdominal pain, nausea and vomiting. These symptoms had been present for about 3 weeks. She was somewhat stuporous and moderately jaundiced. The blood pressure was 88/50. The urine contained bile but no albumin or sugar. The icterus index was 39. The hemoglobin was 72 per cent; erythrocytes, 3,750,000; leukocytes, 13,400. The blood urea nitrogen was 101 mg. per cent. The patient gradually became more stuporous and died on December 15th.

Postmortem examination revealed suppurative cholangitis with multiple abscesses in the liver and the lungs. The kidneys weighed 130 gm. and 140 gm. and their cortices were cloudy. Microscopically the kidneys are normal.

#### *"Hypochloremic" Uremia*

The best known form of extrarenal uremia is that associated with intestinal obstruction. It has long been known that intestinal obstruction gives rise to oliguria, an increase of nitrogenous waste products in the blood, a decrease of blood chlorides and often clinical uremia. It has been shown both clinically and experimentally that injection of a physiological solution of sodium chloride increases the diuresis, relieves the clinical symptoms, decreases the blood urea and non-protein nitrogen and increases the blood chlorides. In fatal cases it has been found that the kidneys are structurally normal. The unfavorable symptoms have generally been referred to the low level of blood chloride; chlorides are lost by the excessive vomiting which commonly accompanies the disease. The renal insufficiency associated with intestinal obstruction is usually called hypochloremic uremia. The chief argument that low blood chlorides are responsible for the uremia is the improvement that follows administration of sodium chloride. Many investigators have tried to explain other forms of extrarenal uremia on the basis of low blood chlorides. Blum and his associates (1929) advanced the theory that urea and other metabolites are retained, when the blood chlorides decrease, in order to maintain the osmotic pressure of the blood.

In recent years, however, convincing evidence has accumulated which shows that the level of the blood chlorides has no causal relation to the level of blood urea or non-protein nitrogen. Dehydra-

tion and increased protein metabolism appear to be the chief causes of extrarenal uremia.

Schiff (1929) gave an extensive survey of the literature on dehydration. He noted that when the water supply of an infant is decreased without diminishing the other constituents of the milk, the child develops toxic symptoms and the non-protein nitrogen of the blood is markedly increased. The plasma chloride is also increased. In pups a low water intake with a normal protein intake causes similar changes and leads to fatty degeneration of the liver, but a low water intake with a protein-free diet does not cause any serious disturbances and there is no increase of non-protein nitrogen in the blood. Kerpel-Fronius (1932) cited a clinical case of dehydration in which the serum chloride was 514 mg. per cent and the non-protein nitrogen 165 mg. per cent. He thinks that azotemia in cases of this type is related to oliguria and dehydration but not to the blood chloride.

Mach and his associates (1934) reported 3 cases of cirrhosis of the liver with marked ascites. The ascitic fluid was removed repeatedly in large amounts, in one patient 92 liters were removed during a period of 7 months. The ascitic fluid had a constant content of chloride, about 600 mg. per cent. During this time the plasma chlorides sank to low levels, 270 to 350 mg. per cent, but the blood urea was not increased in any instance. No better example than this could be cited to show that hypochloremia does not cause azotemia.

Grünwald (1909) found that repeated injections of diuretin in rabbits caused excessive loss of chloride in the urine and that the animals died in coma with a low blood chloride. Bilbao and Grabar (1929) observed that a high blood urea developed along with the low blood chloride but that if salt solution was supplied the animals did not develop azotemia. These investigators attributed the azotemia to hypochloremia. Kerpel-Fronius and Butler (1935) repeated these experiments with diuretin and observed a concentration of the blood as indicated by the increase of the plasma proteins. They also found that administration of water without salt prevented both the azotemia and the concentration of the blood. They attributed the beneficial effects of salt solution to the increased diuresis and not to the effect on the serum electrolytes.

Kerpel-Fronius (1936) removed a large proportion of the chlorine

ions from the blood by intraperitoneal injections of sodium lactate; the sodium ion was not removed. In this experiment the non-protein nitrogen of the blood was not increased. This author gives a summary of the evidence against the view that low blood chloride is a cause of azotemia.

In 5 of our cases (Nos. 17, 18, 22, 24, 25) there was excessive loss of fluid by vomiting, and in No. 23 excessive perspiration probably caused a depletion of body fluid. Although the blood chlorides were not determined these cases presumably belong to the group of so-called hypochloremic uremia. In 4 of these cases the kidneys were normal but in two instances, Nos. 17 and 18, the kidneys were greatly swollen and there is evidently some tubular injury. However, the tubular injury does not seem severe enough to have caused uremia and we must believe that loss of body fluid was chiefly responsible for the decreased urinary output and subsequent retention of nitrogenous products.

In 5 cases (Nos. 13, 14, 16, 26, 27) there was no excessive loss of body fluid although there may have been a decreased fluid intake. There was a severe infection in each of these patients and in the first three definite tubular injury was found at postmortem. However, the structural changes seem too mild to have produced uremia. It is possible that increased destruction of tissue protein, together with an inadequate fluid intake, is responsible for the uremia.

### *The Uremia of Diabetic Coma*

Many investigators have noted that uremia may develop during or after an attack of diabetic coma. Severe oliguria or anuria begins usually 24 to 48 hours after the onset of coma. The patient frequently recovers from coma before the onset of anuria. In 54 cases of diabetic coma Baker found the blood urea nitrogen above 35 mg. per cent in 27; in 15 cases it was above 50 mg. per cent, and in 3 cases above 100 mg. per cent. In a survey of the literature of diabetic coma Fullerton *et al.*, found 6 deaths among 34 patients in whom the blood urea value did not exceed 100 mg. per cent, but there were 14 deaths among 22 in whom the blood urea was above this level.

The azotemia is obviously related to urinary suppression; the problem under discussion for some years is the cause of the decreased diuresis. The urine usually contains a fairly large amount of albumin but this does not indicate a serious renal injury; albumin escapes



through glomerular capillaries and these show no structural changes. If any serious renal injury is present it must be tubular. During the stage of oliguria and nitrogen retention the kidneys can secrete little or no phenolsulphonephthalein, which may mean decreased glomerular filtrate or increased tubular reabsorption.

Several observers have noted that the kidneys are enlarged and cloudy (Warburg, Fullerton *et al.*, Dinkin and Metzger, Metzger, Kraus and Selye); and several have described microscopic tubular lesions, such as granular and vacuolar degeneration and occasional necrotic cells, and have considered the anuria a result of tubular injury (Labbé and Boulin, Fullerton *et al.*, Kraus and Selye). The injury of the kidneys is usually attributed to ketonic acids (Snapper). Metzger studied 17 cases and observed some renal injury but does not think the damage sufficient to cause uremia.

In 2 of our cases the kidneys were greatly enlarged; in 1 of them (No. 19) there was no change but a moderate dilatation of the tubules (Fig. 13), but in the other (No. 20) there was an enormous tubular dilatation (Fig. 14). There is no obstruction in the tubules to explain the dilatation. In this particular case (No. 20, Fig. 14) one is forced to conclude that tubular injury is partly responsible for the azotemia. In our 3rd case (No. 21) the kidneys were entirely normal microscopically. In summary we may say that there is usually some tubular injury in diabetic coma with uremia but that it is seldom sufficiently severe to explain the uremia.

The blood chlorides are sometimes moderately reduced, sometimes normal (Labbé and Boulin, Fullerton *et al.*, Blum *et al.*, Schmitt). There is no evidence in the literature that the uremia of diabetic coma is of the hypochloremic type.

Bulger and Peters found a definite concentration of the blood in diabetic coma, indicated by the increase of hemoglobin and plasma proteins.

The cause of anuria and azotemia in diabetic coma is not clearly established. There is evidence that the following factors may be concerned, *viz.* dehydration, increased endogenous protein metabolism, acidosis with injury of the renal tubules and decreased blood pressure.

#### *Posthemorrhagic Uremia*

Meyler (1935) observed 2 clinical cases of uremia following severe hemorrhage. He reproduced this condition in guinea pigs by re-

peated bleedings. The blood urea rose to high levels but the blood chloride remained at a normal level. The patients as well as the animals secreted a small amount of highly concentrated urine. The animals took very little fluid or food. When given fluid subcutaneously the guinea pigs did not develop uremia. Meyler thinks that there is an enormous destruction of body protein and that the kidneys are unable to excrete the increased amount of nitrogenous waste products because of dehydration.

### *Cerebral Uremia*

It is now well known that various types of injury of the brain may give rise to transitory albuminuria and glycosuria (Morawitz and Schloss). It is seen most frequently after subarachnoid hemorrhage. The albumin and sugar are noted directly after the injury and often both disappear within 48 hours. Acetone is seldom found. Occasionally there is anuria. Morawitz and Schloss reported one patient with a non-protein nitrogen of 134 mg. per cent and another with 162 mg. per cent; both recovered.

We have observed one patient, admitted in coma from carbon monoxide poisoning, who showed albumin and a large amount of sugar in the urine. The condition was diagnosed diabetic coma at first. The postmortem revealed normal kidneys. Another patient who sustained a traumatic injury of the brain was first seen in coma with albuminuria and glycosuria, but there was no acetonuria. The postmortem examination revealed bruising of the frontal lobes of the brain but no subarachnoid hemorrhage. The kidneys were normal.

A third patient with a subarachnoid hemorrhage developed a non-protein nitrogen of 160 mg. per cent but subsequently recovered.

Cerebral glycosuria is attributed to injury of the sugar-regulating center in the floor of the fourth ventricle. There is no satisfactory explanation for cerebral albuminuria and uremia. It has been suggested that uremia is due to oliguria induced by spasm of the renal vessels. Low blood pressure is sometimes a possible factor but azotemia may develop with a normal or an increased blood pressure.

In summary it may be said that several factors may be concerned in the development of extrarenal uremia, the most important of which are dehydration and increased endogenous protein metabolism. It is unlikely that a decrease of blood chlorides is a cause of azotemia.

## SUMMARY

One hundred and ten cases of clinical acute nephritis have been classified in accordance with the structural changes in the kidneys.

There were 31 cases of uncomplicated acute glomerulonephritis and 20 cases in which the nephritis was associated with another disease.

Obstruction of the glomerular circulation is usually due to endothelial proliferation, but in a few instances it is due partly or largely to epithelial crescents, intracapillary thromboses, thrombosed arterioles or polymorphonuclear leukocytes.

In the normal glomerulus and in subclinical glomerulonephritis it may be seen that all the capillaries of the lobules are completely invested with a basement membrane, but in clinical glomerulonephritis the capillaries within the lobule become fused together and their inner basement membranes split to form the characteristic intracapillary fibers. The lesions are all intracapillary; the appearance of "intercapillary" lesions is due to the persistence of portions of the capillary lumens in the peripheral parts of the affected lobules.

In 5 cases uremia was due to numerous massive lesions of the embolic type, in the absence of endocarditis.

Eleven cases are reported in which the outstanding symptoms were septicemia, hematuria and uremia. This is called the hemorrhagic type of glomerulonephritis. The blood escapes through ruptured glomerular capillaries and uremia is due to obstruction of the tubules by masses of red blood cells or hemoglobin.

Albuminuria, hematuria and edema of renal origin are evidences of glomerular injury; tubular disease is evidenced by oliguria and anuria.

In rare instances acute uremia is due to multiple thromboses of small renal arteries.

The most frequent form of tubular nephritis is that associated with mercuric chloride poisoning but there are occasional instances of tubular disease due to other causes.

The acute uremia following transfusion with incompatible blood is due chiefly to obstruction of the collecting tubules by casts of hemoglobin.

There is a group of cases in which uremia seems to be partly of extrarenal origin and partly due to distention of the convoluted tubules with minor degenerative changes in their lining cells.

In purely extrarenal uremia the kidneys are normal and the azotemia is due chiefly to dehydration and to increased destruction of protein. Decrease of blood chloride is apparently not a cause of azotemia.

The azotemia of diabetic coma is due in part to tubular injury in some instances.

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## DESCRIPTION OF PLATES

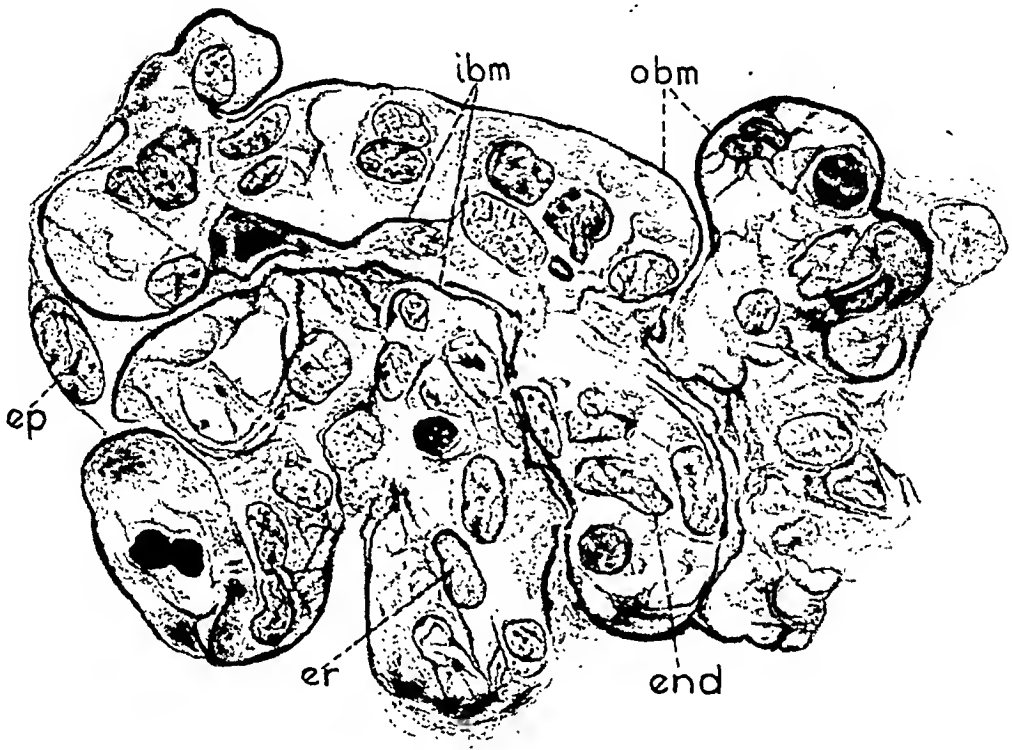
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### PLATE 85

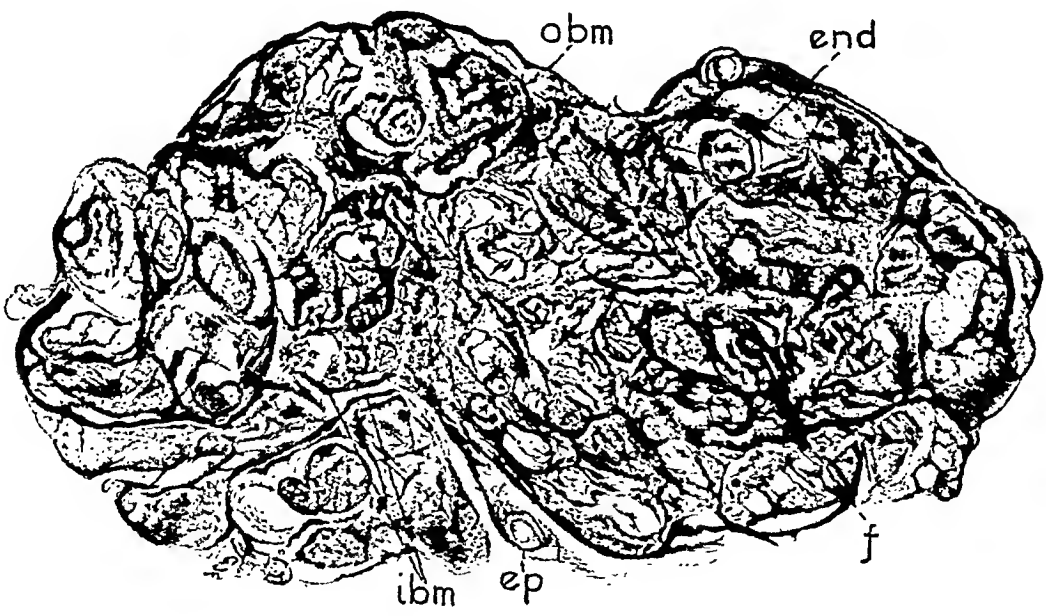
Fig. 1. (Table I, No. 3.) Lobule of a glomerulus showing an early stage of acute proliferative glomerulonephritis. The outer basement membrane (o b m) of the capillaries is intact but the inner basement membrane (i b m) has begun to break up into intracapillary fibers.

End. = endothelial cell; ep. = epithelial cell; er. = erythrocyte. Mallory-Heidenhain stain.  $\times 1200$ .

FIG. 2. (Table I, No. 10.) Lobule of a glomerulus showing a more advanced stage of acute proliferative glomerulonephritis than that illustrated in Figure. 1. The capillaries composing the lobule are fused into a compact mass. The inner basement membranes have broken up into intracapillary fibers (f). Other lettering as in Figure 1. Mallory-Heidenhain stain.  $\times 1200$ .



1

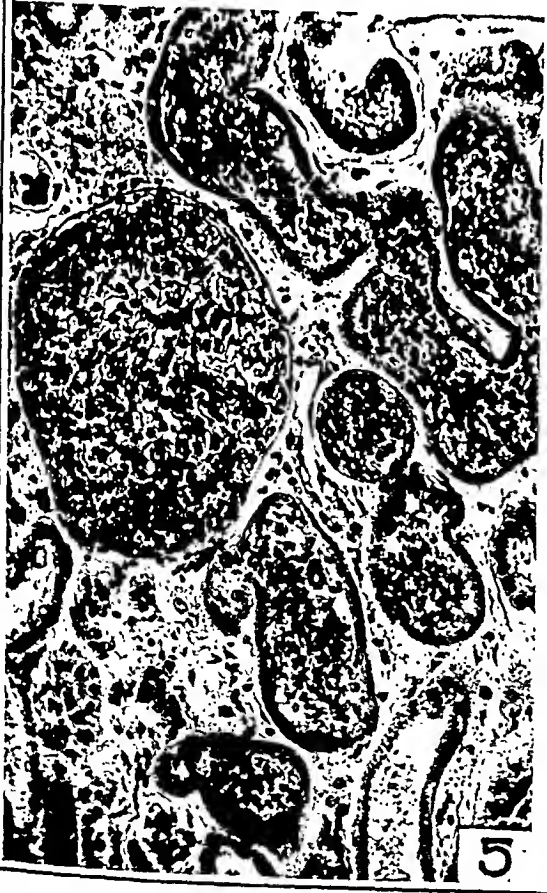


2



PLATE 86

- FIG. 3. (Table I, No. 52.) Glomerulus showing thrombosis of nearly all of its capillaries.  $\times 350$ .
- FIG. 4. (Table I, No. 64.) Glomerulus showing an embolic type of lesion. Nearly all the glomeruli showed lesions similar to this. There was no endocarditis.  $\times 350$ .
- FIG. 5. (Table I, No. 75.) Hemorrhagic type of glomerulonephritis. Note that the capsular space and the tubules are distended with blood.  $\times 250$ .
- FIG. 6. (Table I, No. 72.) Hemorrhagic type of glomerulonephritis. Note collecting tubules distended with casts composed largely of hemoglobin.  $\times 300$ .

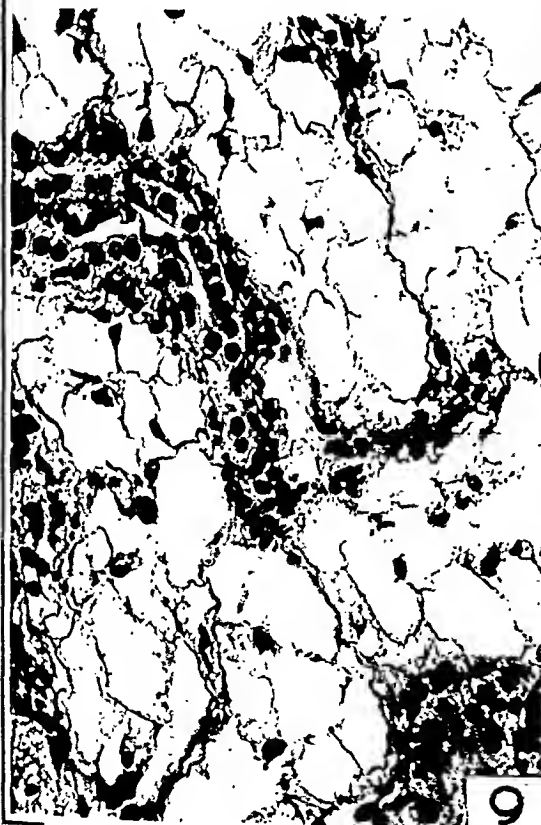


Bell

Clinical Acute Nephritis

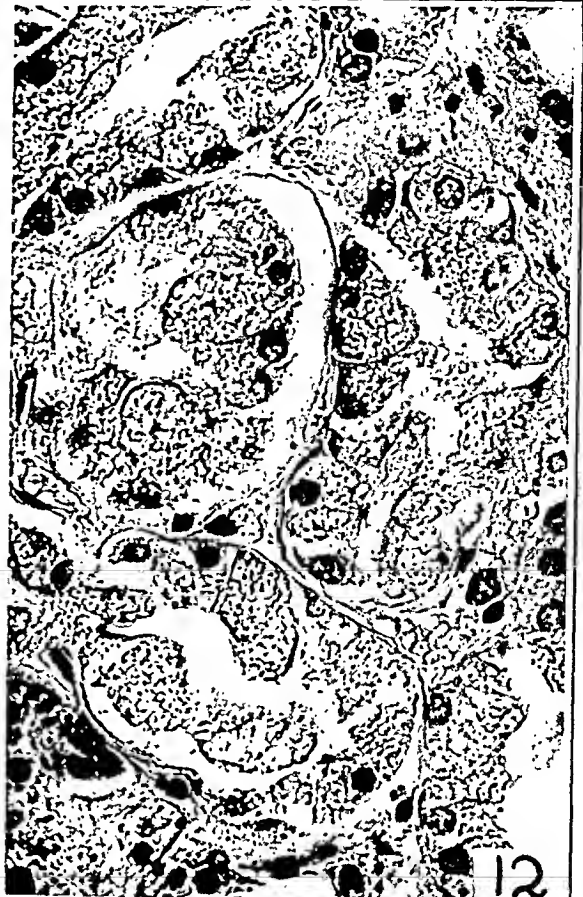
PLATE 87

- FIG. 7. (Table I, No. 4.) From a case of mercuric chloride poisoning of 10 days duration. Note that the cells of some of the convoluted tubules are largely destroyed; only a thin basal zone of cytoplasm persists.  $\times 400$ .
- FIG. 8. (Table I, No. 7.) From a case of mercuric chloride poisoning of 16 days duration. Note that the lining cells of the convoluted tubules are flattened, and that they take the basic stain. The normal granulation is absent. These are interpreted as regenerated epithelial cells.  $\times 350$ .
- FIG. 9. (Table I, No. 9.) Tubular nephritis. Note the extreme hydropic degeneration of the convoluted tubules.  $\times 350$ .
- FIG. 10. (Table I, No. 11.) Obstructive tubular disease from blood transfusion. Area from the cortex showing dilation of tubules and interstitial edema.  $\times 200$ .



## PLATE 88

- FIG. 11. (Table I, No. 11.) Obstructive tubular disease from blood transfusion. Area from the medulla showing collecting tubules obstructed by casts composed of hemoglobin.  $\times 350$ .
- FIG. 12. (Table I, No. 14.) Extrarenal uremia with some tubular injury. The cells of the convoluted tubules are filled with small vacuoles but there are no necrotic cells.  $\times 450$ .
- FIG. 13. (Table I, No. 19.) Extrarenal uremia with some tubular injury. The kidneys weighed together 600 gm. All the cortical tubules are dilated but only a few casts are found. There is no necrosis.  $\times 350$ .
- FIG. 14. (Table I, No. 20.) Extrarenal uremia with some tubular injury. Death from diabetic uremia. The kidneys weighed together 460 gm. All the tubules are enormously dilated and lined by pale flattened epithelium. No definite necrosis is seen. There are only a few casts.  $\times 350$ .







## PARATHYROID HYPERPLASIA IN CHRONIC RENAL INSUFFICIENCY \*

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In contrast with the present universal recognition of the effects of parathyroid abnormalities on the skeletal system relatively little attention has been devoted to the close interrelation of the kidneys and the parathyroid glands. Yet there is reason to believe that instances of the latter are far commoner than the former. For this relation appears to work in either direction. Hyperparathyroidism, if sufficiently long continued, will eventually lead to renal insufficiency because of calcium deposits in the kidney parenchyma, while primary renal insufficiency of a severe and prolonged character will produce parathyroid hyperplasia and — if we accept as evidence the histological alterations in the bones and Shelling and Remsen's<sup>1</sup> parathormone assay of the blood — true functional hyperparathyroidism. Starting, therefore, with either primary lesion a patient may reach an apparently similar end-stage of combined hyperparathyroidism and renal insufficiency. The possibility, therefore, of distinguishing between primary and secondary changes in the parathyroid gland has practical as well as theoretical significance.

In a previous paper describing the pathology of the parathyroid glands in 25 cases of hyperparathyroidism<sup>2</sup> a classification was proposed that divided the lesions sharply into two groups, one in which the changes were restricted to one gland, part of a gland, or occasionally two glands (presumably neoplasia), and a second group in which diffuse hypertrophy and essentially uniform histological changes in all the glands occurred — a condition we classified as hyperplasia. We described furthermore two types of hyperplasia; one in which all the cells are unusually large with clear cytoplasm and nuclei uniformly oriented to the base of the cell (true wasserhelle cells), and another type in which all the glands are composed, except for a scattering of oxyphil cells, of closely packed, normal sized chief cells. A single case of this chief cell type of hyperplasia was

\* Received for publication May 3, 1937.



presented and though it was recorded as being found in a patient with chronic renal insufficiency this relation was not stressed.

In the two and a half year period since our former report 12 new cases of hyperparathyroidism have been seen at the Massachusetts General Hospital. Ten of these were single adenomas, 1 was a wasserhelle hyperplasia, and 1 a chief cell hyperplasia again associated with a long-standing renal insufficiency. No case has been seen that failed to fit readily into the previously described categories and the additional clinical evidence that has accumulated — the absence of a single recurrence of symptoms in the adenomatous group contrasted with several recurrences in the hyperplastic group — strongly substantiates the validity of our classification.

The observation of a second case of pronounced chief cell hyperplasia in association with osteitis fibrosa and chronic renal insufficiency served to emphasize the distinction between the wasserhelle type of hyperplasia — to be called hereafter for the sake of simplicity "primary hyperplasia" even though we realize that it cannot be truly a primary disease of the parathyroids — and the entirely different hyperplasia that is secondary to chronic renal insufficiency. This stimulus led us to study the parathyroids in a group of 29 cases of chronic renal insufficiency and also to search for secondary hyperplasia in other disease states. The present report is a résumé of this study. Our material consists of 2 cases (1 previously reported) of chronic renal insufficiency with bone lesions indistinguishable from those of hyperparathyroidism, 12 cases of chronic glomerular nephritis without bone lesions, 15 cases of renal insufficiency of other types, and an assortment of 9 other cases in which secondary hyperplasia was found. We have included also for purposes of contrast and because cases of this type are still not numerous an additional case of so-called primary hyperplasia.

#### REVIEW OF THE LITERATURE

The fact that one or more parathyroid glands may be enlarged in chronic glomerular nephritis or in any form of chronic renal insufficiency is not an original observation. In a paper published in 1935 Pappenheimer and Wilens<sup>3</sup> compared the weights of the parathyroids of normal individuals with those of a group of nephritics and showed quite conclusively that the latter were 50 to 100 per cent heavier. In a later communication Jarrett, Peters and Pappen-

heimer<sup>4</sup> reported the production of enlargement of the parathyroids in rats by a total nephrectomy on one side and partial cauterization of the other kidney. They did not, however, discuss the histological changes in the glands and descriptions of the latter are comparatively rare.

More recently Gilmour and Martin<sup>5</sup> published an exhaustive statistical study of the weights of the parathyroids in a series of 527 cases of varied diseases. They calculated the weight of the parenchyma as distinct from the fatty stroma and from their figures the parenchymatous portions of the glands from the nephritics and renal disease groups were over 60 per cent heavier than the normals. Many of the cases included in their renal group, however, were probably not cases of chronic renal insufficiency so that this figure would certainly have been much higher had cases of the latter only been selected. Here also no histological studies were reported.

In 1905 MacCallum<sup>6</sup> reported a case of chronic glomerular nephritis in which he found one enlarged parathyroid and two apparently normal glands. Bergstrand<sup>7</sup> in 1921 reported 10 cases of parathyroid enlargement associated with chronic renal insufficiency. In 7 of these cases there was a diffuse chief cell hyperplasia of all the glands. In some the gross enlargement was especially marked in one or two of the glands of an individual case but similar histological changes were present in all of the glands. The remaining 3 cases showed enlargement of only one gland, the others being normal in size and structure. In one of this group a rim of normal parathyroid tissue about an apparent adenoma was described. In none of these cases were any clinical data recorded and there was no mention of examination of the bones beyond the statement that one of the cases showed osteoporosis.

The numerous case reports of renal rickets — a disease of children characterized by chronic renal failure usually due to an anomaly in the urinary tract with secondary demineralization of the skeleton — might be expected to provide considerable information relative to the parathyroids. Unfortunately, however, they have not been examined or at any rate described in most of the cases of renal rickets that have been reported. Mitchell<sup>8</sup> in 1930 abstracted 78 cases of renal rickets but makes no mention of the parathyroids in any of them. We have checked most of these case reports and found that many were purely clinical and that in those where autopsies

had been performed examination of the parathyroids was not recorded.

Langmead and Orr<sup>9</sup> in 1933, Smyth and Goldman<sup>10</sup> in 1934, and Price and Davie<sup>11</sup> in 1937 reported cases of renal rickets with diffuse parathyroid enlargement. In Hubbard and Wentworth's<sup>12</sup> case of metastatic calcification and osteitis fibrosa associated with chronic nephritis two parathyroid glands were identified and were said to be hyperplastic though no microphotographs or microscopic descriptions were given.

Shelling and Remsen<sup>1</sup> reported a case of renal rickets in which increased amounts of parathyroid hormone were demonstrated in the blood and in which four enlarged parathyroid glands were found. The description and microphotographs of the glands are identical with our cases of secondary hyperplasia.

## CASES OF PARATHYROID HYPERPLASIA

### A. PRIMARY HYPERPLASIA

CASE 26\*: A. T. (35-534), a housewife, aged 57 years, was admitted Jan. 21, 1935. Following three attacks of renal colic she passed a urinary calculus in March, 1931. One month later her right kidney was removed at another hospital. In the summer of 1934 she experienced almost daily attacks of dull pain in the left lower back radiating at times to the groin. During the 6 months prior to admission she had vague aches and pains throughout her body and had lost 15 pounds. Her weight on admission was 83 pounds.

X-rays showed generalized skeletal decalcification with several areas of diminished density in the skull that suggested multiple myeloma. The serum calcium ranged between 14.46 and 16.38 mg. per cent, and the serum phosphorus from 2 to 2.91 mg. per cent. The phosphatase was 4.84 Bodansky units. A bone biopsy showed the changes characteristic of hyperparathyroidism. Renal function tests showed normal excretion by the remaining kidney.

On Feb. 9, 1935, the parathyroid glands were exposed and found to be enlarged (Fig. 1). Both superior and the right inferior glands were entirely removed. Approximately three-fourths of the left lower gland was resected, leaving residual tissue estimated at 0.225 mg. with a good blood supply.

*Gross Description: (Left Upper):* A somewhat flattened, soft, triangular shaped, slightly fluctuant mass 5 by 2 by 3.5 cm. which weighed approximately 6 gm. The surface of the upper two-thirds was purplish red, while that of the lower third was yellowish brown. On section the upper two-thirds was made up of many small cystic

\* This is the 26th case in the Massachusetts General Hospital series and the number is used here to correspond with any reference to it in previous or subsequent articles.

cavities 1 to 3 mm. in diameter, from which dark red blood exuded. The lower third was homogeneously yellowish brown.

(*Left Lower*): A smooth surfaced, yellowish brown, encapsulated soft mass measuring 1.5 by 1 by 0.8 cm. which weighed 0.86 gm. The cut surface was uniformly yellowish brown. A portion of this gland was not resected.

(*Right Upper*): An irregularly shaped, encapsulated, lobulated, soft, smooth surfaced mass which measured roughly 3 by 2 by 1 cm. and weighed 3.75 gm. The surface of some parts of the gland was dark purplish red and other parts yellowish brown. One pole was composed of three pseudopod-like projections 1.5, 1 and 1 cm. in length and approximately 1.5 to 1 cm. in diameter. The cut surface was reddish to yellowish brown.

(*Right Lower*): Similar to the left lower, measured 1.8 by 0.7 by 0.3 cm. and weighed 0.59 gm.

*Microscopic Examination*: A detailed description of this type of hyperplasia was given in our previous paper\* but is so perfectly applicable to this case that it will be repeated here in order to compare it with the hyperplasia secondary to chronic renal insufficiency. All the glands have the same appearance. "There is only one type of cell throughout, the wasserhelle cell, which is polyhedral in shape, sharply demarcated by a thin eosinophilic membrane, and varies from 10 to 40  $\mu$  in diameter, averaging 15 to 20 (Fig. 3). Many of the cell boundaries are broken, with resultant fusion, similar to the fusion of alveoli in pulmonary emphysema. In contrast with the variability in the size of the cells, the nuclei, though often multiple, are all approximately the same size, averaging about 8  $\mu$  in diameter. They are round to slightly ovoid in shape, sharply outlined, moderately hyperchromatic, with an eccentrically placed nucleolus. As a rule the nuclei are located in the end of the cell that is contiguous to the stroma. This produces a characteristic pattern which resembles branches of berries (Fig. 2). The cytoplasm is clear except for a little, light pink-staining granular material. Many of these tiny granules are glycogen deposits. Similar granules are present within the nuclei. There is no fat, except for a rare droplet in the stroma. The low power appearance of the histological sections is so similar to that of clear cell renal carcinomas that distinction would be difficult if the source were not known. The stroma is composed of thin,

\* Case 15, pages 7 and 8.

fibrous connective tissue bands containing a moderate number of connective tissue cells and relatively few blood vessels. These bands surround small and large groups of cells, producing a pseudoglandular effect. This effect is further emphasized by the position of the nuclei, as mentioned above. Occasionally a true single layered alveolus is seen. No oxyphil or chief cells are found. There are no mitoses."

## B. HYPERPLASIA, SECONDARY TO CHRONIC RENAL INSUFFICIENCY

### *Group 1. With Secondary Osteitis Fibrosa and Ectopic Calcification \**

F. L. S. (7964)†, a 45 year old American, entered the hospital on Nov. 5, 1935, complaining of painful nodules in his fingers. Twenty-three years before admission he was studied at another hospital for generalized dropsy, was told he had incurable Bright's disease, and was placed on a salt-free, meatless diet. From that time until 2½ years before admission the patient was in apparent good health with no return of edema. Then he began to have itching of the skin, nocturia, and later swelling of his fingers. The positive findings in the physical examination were precordial systolic and diastolic murmurs, cystic swellings on the right forefinger, and firm and tortuous peripheral vessels. The blood pressure was 165/90.

Examination of the urine showed a specific gravity from 1.006 to 1.012, a large trace of albumin and a sediment containing an occasional red blood cell and white blood cell. A phenolsulphonephthalein test showed less than 15 per cent excretion at the end of 1 hour. Examination of the blood showed a red blood cell count of 3,800,000 with a hemoglobin of 65 per cent, and a white blood cell count of 12,000, 63 per cent polymorphonuclears. The non-protein nitrogen was 120 mg. The serum calcium was 10.10 mg. per cent, serum phosphorus 7.92 mg. per cent, and phosphatase 9.36 Bodansky units. The serum protein was 4.9 gm. per cent. X-ray examination showed masses of homogeneous calcification surrounding the proximal interphalangeal joints and along the phalanges of the right second, third and fourth fingers. Both elbows and the acromioclavicular joints showed similar calcified masses. The bones of the pelvis showed slight decalcification. The skull was riddled with small areas of decalcification. In all the films the large and small blood vessels showed marked arteriosclerosis with calcification, some of them definitely of the Mönckeberg type. A pyelogram showed extremely small kidneys.

While on the ward during the 3rd week he was suddenly seized with severe pain between the shoulder blades and later the pain was localized in the left anterior chest and upper abdomen. He developed bundle branch block; the blood pressure fell to 60/50 and he died 5 hours later.

\* One similar case was reported in our previous paper,<sup>2</sup> Case 23A, page 12.

† A clinical discussion of this case has been reported separately by Dr. Fuller Albright in the *Tr. A. Am. Physicians*, 1936, 51, 199-212. This case has also been reported in the Case Records of the Massachusetts General Hospital, *New England J. Med.*, 1936, 214, 320-325.

Postmortem examination showed in addition to the parathyroid enlargement a marked chronic glomerular nephritis, metastatic calcification around joints, calcification of the coronary, renal and splenic arteries, coronary occlusion, and rheumatic heart disease with mitral stenosis. Death was due to coronary thrombosis. Sections of the various bones showed a mild to moderate degree of decalcification and osteitis fibrosa (Fig. 9). The latter was indistinguishable from the bone lesions in primary hyperparathyroidism, except that no cysts were present.

*Gross Description:* All four parathyroid glands were in their normal position. They were all enlarged, firm, yellowish white, and somewhat lobulated (Fig. 4). The cut surface was uniformly yellowish white and smooth. (Note the absence of any brown color, a finding that is present in normal glands, adenomas, and primary hyperplasias.) In one gland a calcified nodule was found. The right lower was the largest, measured 2 by 1.5 by 1.5 cm., and one-half of it weighed 2 gm. From the weight of this half the weights of the glands were estimated as: right lower 4 gm., left lower 3 gm., and the upper glands 2 gm. each.

*Microscopic Examination:* The picture here is almost identical with that of the case of chief cell hyperplasia described in our previous paper. None of the architectural features of a normal parathyroid gland can be made out. Instead of anastomosing columns of epithelial cells separated by bands of fibrous stroma containing large fat cells, the tissue consists of an almost solid sheet of epithelial cells punctuated only by vascular channels at rather regular intervals with very scant fibrous stroma chiefly limited to the adventitia of the blood vessels (Fig. 8). Practically no fat cells are present. The structure is by no means uniform, however, for even with low power it is evident that there are circumscribed islands of varying size often encapsulated with a delicate band of collagen. Either a peculiarity of the arrangement of the cell cords or a consistent variation in the cells themselves serves to mark the islands clearly from the surrounding tissue (Figs. 5 and 6). Throughout the great majority of the gland the predominant cell is the chief cell, a trifle more vacuolated than normal, but with a cytoplasm by no means totally clear. Within each of the circumscribed islands which have been mentioned the cells tend to be very similar in appearance but between one island and another there may be marked variation

(Fig. 7). In one, for instance, the cells are uniformly large and tend to show a high degree of vacuolization sometimes approaching the appearance of the wasserhelle hyperplasia, though the largest cells observed are only half the size of those characterizing that condition. In this same island numerous small acini are found with a granular serous secretion in their lumens. In other islands the cells show less than normal vacuolization of the cytoplasm but the nuclei are a little large and distinctly hyperchromatic. A columnar architecture (observed throughout most of the gland) is rendered prominent by a widening of the vascular channels and an increase in the collagenous stroma. In still another localized area cystic spaces often filled with red cells but without an endothelial lining are seen. This appearance is one we have noted in chief cell adenomas. Occasionally within one of these islands smaller secondary islets can be made out. About the periphery of the larger islands a zone of smaller, more compactly arranged cells is suggestive of compression from the growth of the island itself.

Scattered throughout all the gland singly, in small clusters, and in large nodules, are cells with oxyphil granules in their cytoplasm (Fig. 7). The cells vary in diameter from that of a normal chief cell to three times this size; the granules may be sparsely scattered or so densely packed as to make the cytoplasm apparently homogeneous; there may be extensive vacuolization or there may be none. The nuclei vary from vesicular to pyknotic. In short, the entire gamut of transition stages from chief cell to fully developed oxyphil is present.

A review of the histological features of Case 23A in our former paper, the chief cell hyperplasia with chronic pyelonephritis and osteitis fibrosa, shows that they are in all essentials similar to those of this case. The hyperplasia is diffuse throughout all the glands, the predominant cell is the chief cell; rare acinar formation and occasional wasserhelle cells are observed, whereas transitional and fully developed oxyphils are very numerous. It likewise shows clearly developed islands in which one or another type of cell predominates and around which the surrounding gland tissue seems compressed.

#### *Group 2. Chronic Glomerular Nephritis Without Secondary Bone Involvement*

This group is comprised of 12 successive cases of chronic glomerular nephritis in which the parathyroids were available for study.

The pertinent clinical and postmortem findings are summarized in Table I. The duration of symptoms, as far as this can be estimated from the clinical histories, has been given, though a glance at the weights of the kidneys makes it apparent that the renal disease must

TABLE I  
*Chronic Glomerular Nephritis*

No.	Age	Duration	Non-protein nitrogen	Calcium	Phosphorus	Parathyroid			Combined weight of kidneys
						No.	Size	Weight	
	yrs.	mos.	mg./%	mg./%	mg./%		mm.	mg.	gm.
1	24	11	200	..	..	2	Normal	..	120
2	30	6	300	..	..	4	8×4×3, 15×8×4 12×5×2, 14×10×4	105, 293 125, 340	75
3	32	8	127	6.35	5.70	4	12×8×5, 3 normal	863	125
4	42	12	180	..	..	4	15×1×4, 8×6×3 2 normal	..	60
5	19	18	205	..	..	4	12×6×3, 9×4×3 10×8×4, 11×8×2	630	100
6	52	12	120	..	..	4	Normal	..	270
7	30	7	190	8.44	8.40 14.42	3	Sl. enlarged	..	150
8	56	30	220	5.91	16.0	4	8×5×4, 3 normal	..	150
9	25	11	105	8.94	5.32	3	1 sl. enlarged 2 normal	..	170
10	26	15	265	8.09	15.65	4	8×5×2, 8×4×3 10×5×2, 7×5×3	360	225
11	26	36	250	7.52	12.13	4	Each 1.2×0.5×0.3	846	120
12	39	16	250	..	..	4	1 sl. enlarged 3 plump	..	240

in most instances have been far more chronic than the symptoms would indicate. They are evidently as a group, however, of distinctly shorter duration than the cases in the preceding group and this undoubtedly accounts for the absence of bone pathology. The vertebral bone marrow was examined in all these cases and found to be negative, except in one which showed slight degrees of bone re-



sorption without, however, any evident fibrosis. The levels of blood calcium and blood phosphate where available are worthy of note. Without exception the calciums are low and the phosphates moderately to markedly elevated, in contrast with the exactly reverse condition — high serum calcium and low serum phosphorus — found in primary hyperparathyroidism.

In this group of cases all but 2 showed gross enlargement of one or more of the glands. In 5 cases enlargement of all the glands was noted (Fig. 10), in 3 cases it was evident in only one, and in 1 case two were enlarged and two were normal. These gross findings are very similar to those of Bergstrand.<sup>7</sup>

With microscopic examination, however, our findings become different. We have found in all of the glands of every case in this group what we consider definite evidence of hyperplasia. Since this hyperplasia is admittedly in some of the cases rather slight, it becomes necessary to examine the criteria on which a diagnosis of hyperplasia can be made.

Let us recall for a moment the architectural features of the normal gland. It possesses a structure rather common among the endocrine glands of anastomosing columns of epithelial cells surrounding vascular spaces which are usually a little wider than ordinary capillaries and approach the character of sinusoids. But in the parathyroids this columnar structure is more complicated than in the other endocrine glands in that it is doubled. Cords 2 to 4 cells wide anastomose about the smaller vascular channels but these are in turn grouped into larger columns 4 to 20 cells in width, which in turn anastomose about the larger vessels and the fibrous stroma of the organ, a stroma, moreover, that is unique among the endocrine glands in that large fat cells are normally present in it in considerable numbers at all times after puberty.

With progressive grades of hyperplasia these fat cells steadily decrease in number and eventually may even disappear. They appear to behave essentially like the fat cells of the bone marrow, modestly giving way to the more important parenchymal cells as need arises. This would explain why some hyperplastic glands are not increased in size, *i.e.* the hyperplasia has progressed only to the point of fat displacement.

The proportion of fat cells to parenchyma, although a very important aid, is by no means an entirely satisfactory criterion since

the "normal" proportion of fat is far from constant, varying significantly with age. Probably the next most useful yardstick is the character of the epithelial columns. Although under normal conditions these show a wide range of variation from 4 to 20 or more cells in thickness, the majority run from 4 to 12 and the thicker ones are found only in limited portions of a gland. With hyperplasia, more and more of the columns are found in the upper ranges and solid sheets of cells without discernible columnar arrangement appear (Fig. 11). Not merely the fat cells but even the fibrous stroma tends to disappear. This widening of the epithelial columns and progressive diminution in the fat and even the fibrous stroma produce a decided compactness of the tissue which is obvious at a glance with low magnification. Though small compact areas may be found in some presumably normal glands, the extension of this appearance to any large proportion of the gland certainly indicates hyperplasia.

Finer cytological details have proved of relatively little help. In the early stages of hyperplasia a tendency to increased vacuolization and simultaneously an increase in the glycogen content, as judged by Best's carmine reaction, is apparent, but with the more marked degrees the cells tend to become smaller once more and most of them revert to the typical chief cell appearance. The search for mitotic figures has, as in the case of the adenomas, proved disappointing. Even in the most extreme hyperplasia — a hundredfold increase in the amount of parathyroid tissue — a 20 minute search with an oil immersion lens has failed to reveal one.

For the sake of clarity the following tabulation of our criteria for the diagnosis of chief cell (secondary) hyperplasia seems worth while.

### *Criteria for the Diagnosis of Chief Cell Hyperplasia*

#### *Gross Appearance:*

- (A) *Size:* Characteristically slight to moderate enlargement of all glands rarely reaching the size of the usual adenoma or primary hyperplasia; however, marked variation is not infrequent in the size of the individual glands of a given case and one or all of them may even be normal in size.
- (B) *Color:* The glands tend to be a creamy gray rather than an orange-brown.

- (C) *Consistence*: The glands are firmer and much less pliable than the normal gland, the adenoma or the primary hyperplasia.

*Microscopic Appearance*:

(A) *Low Power*

1. Uniformity of all glands in a given case.
2. Absence or marked decrease in intercellular fat cells.
3. Increase in number of cells as shown by widening of the epithelial columns.
4. Development of compact areas in which columnar arrangement is no longer distinguishable.
5. Tendency to acinar arrangement in the more advanced cases.
6. Uniformity of the whole gland except in the more advanced cases where there is a tendency to adenomatous-like and papillary formations without real encapsulation.

(B) *High Power*

1. Cells are *normal sized* chief cells, unlike the adenoma or primary hyperplasia.
2. Tendency to vacuolization of the cytoplasm in less severe cases producing slight cell enlargement.
3. No mitoses or hyperchromatism.
4. Oxyphil cells more numerous than expected for age of individual (Fig. 12).
5. Glycogen content slightly higher than adenoma or primary hyperplasia.

Judged on such criteria none of the 12 cases in this group fails to show some evidence of hyperplasia, though in 2 of them it is but slight — not greater than that to be observed in the succeeding group of cases. In the individuals in the present group where gross enlargement was present in one gland only, evident hyperplastic changes have been observed microscopically in the normal sized glands as well and except in degree there has been no difference between the small and the large glands. As compared with the preceding cases with bone involvement, the picture also differs only in degree, the persistence of a few fat cells, and a slightly less degree of island formation and acinar arrangement.

*Group 3. Mild Degrees of Secondary Hyperplasia*

In a review of the microscopic slides of the parathyroid glands removed from 300 routine autopsy cases (excluding chronic glomerular nephritis), we were able by the use of the criteria listed above, without knowing the anatomical or clinical diagnoses, to select 23 cases in which we believed there was definite hyperplasia. Fourteen of these cases showed, both clinically and pathologically, evidence of some degree of renal insufficiency. Eight of the 14 cases were on the Genito-Urological Service for pyelonephritis; the 9th was a case of congenital polycystic kidneys; the 10th, multiple myeloma with renal involvement; and the last 4 were cases of vascular nephritis. One of the latter had malignant nephrosclerosis of one kidney, the other kidney being atrophic with its pelvis and ureter obstructed by gritty calcified material. The pertinent findings in these cases are given in Table II.

The parathyroid glands removed from the case of polycystic kidneys were surprisingly normal in size. Only two were found and unfortunately they were not weighed. Both of these, however, are alike histologically. There was almost complete disappearance of the intercellular fat cells. Except for occasional single and small groups of oxyphil cells, the glands were composed of chief cells of the transitional wasserhelle type, *i.e.* cells that are on their way to wasserhelle cells. These cells were larger than the normal chief or even oxyphil cell, but did not reach the size of the large wasserhelle cell seen in primary hyperplasia.

One of the 9 cases of pyelonephritis, No. 15 in Table II, showed evidence of very early osteitis fibrosa with characteristic dissecting resorption of the spongiosa, as described by Schmorl<sup>13</sup> and Jaffe.<sup>14</sup> This case might, therefore, fit into the group of renal rickets or our Group 1, but because of the mild degree of bone change, when compared to the other 2 cases, we have preferred not to include it in the renal osteitis fibrosa group.

The remaining 9 cases were not cases of renal insufficiency. Three, however, had bone disease which probably accounts for the secondary parathyroid changes — rickets, metastatic carcinoma and Paget's disease. Whether the latter can really be responsible for the parathyroid hyperplasia is questionable. Two of the cases had duodenal ulcers. One of these, however, showed pituitary basophilism which may account for the parathyroid changes. One of the

TABLE II  
Mild Degrees of Secondary Hyperplasia

No.	Age	Duration	Non-protein nitrogen	Phenol-sulphone-phthalein	Calcium	Phosphorus	Parathyroid		Anatomical Diagnoses
							No.	Size	
I	57 yrs.	3 1/2 yrs.	mg./% 36-135	% ..	mg./% ..	mg./% ..	3	mm. 2 normal 1 sl. enlarged	Carcinoma of bladder. Pyelonephritis
2	62	5	280	5	..	..	2	1 1/2 normal	Prostatic hyperplasia. Pyelonephritis
3	57	12	81	..	9.79	3.38	4	Normal	Renal stones. Pyelonephritis. Carcinoma of bladder
4	52	31	128	..	..	..	1	Twice normal	Renal stones. Pyelonephritis
5	59	20	..	0-5	9.76	7.90	4	Slight ++	Atrophy of left kidney. Malignant nephrosclerosis of right. Nephrolithiasis
6	49	3	86	Trace	5.2	9.1	2	Normal	Polycystic kidneys
7	35	10	210	5	8.48	11.76	2	1 normal, 6x4x3	Benign vascular nephritis
8	30	6 1/2	88	Trace	..	..	2	1 sl. enlarged. 1 normal	Pyelonephritis
9	58	1/2	53	25	9.40	4.96	4	All 8x5x3	Multiple myeloma
10	44	8	66	0-3	..	..	3	Normal	Hydronephrosis. Neurogenic bladder
11	39	22	220	..	8.81	15.66	3	Twice normal	Malignant vascular nephritis
12	32	10	80	..	7.7	6.4	1	Normal	Benign vascular nephritis
13	54	1 1/2	31	..	..	..	2	Normal	Pyelonephritis
14	27	3 1/2	20	25	..	..	1	Normal	Nephrolithiasis. Pyonephrosis
15	17	3	150	5	..	..	4	Twice normal	Congenital anomalies of bladder and ureters

remaining 4 cases had malignant hypertension with slight chronic vascular nephritis, but with a non-protein nitrogen of only 24 and a phenolsulphonephthalein of 40 per cent, it was felt that this case did not belong with the group of renal insufficiency cases. The remaining 3 cases — bronchial asthma, rheumatic heart disease, and metastatic carcinoma of the lung from a carcinoma of the cecum — showed nothing in the kidneys that could be responsible for the parathyroid changes. Table III gives abstracts of the cases in this group.

TABLE III  
*Hyperplasia in Non-Renal Cases*

No.	Age	Parathyroid		Anatomical Diagnoses
		No.	Size	
1	yrs. 12	1 (operation)	Normal <sup>mm.</sup>	Rickets
2	36	..	...	Oat cell carcinoma of lung with metastases to bones
3	64	4	Normal	Paget's disease of bone. Glioma
4	67	3	2 normal. 1 (8×5×4—80 mg.)	Duodenal ulcer, gastritis, pituitary basophilism
5	55	3	Normal	Duodenal ulcers for 25 yrs. Had taken soda bicarbonate for yrs.
6	42	3	Normal	Hypertension. Chronic vascular nephritis, slight
7	65	1	Normal	Rheumatic heart disease
8	64	3	Normal	Bronchial asthma
9	33	4	Normal	Metastatic carcinoma of lung from cecum

On gross examination most of the glands in this group showed very slight but definite enlargement. The microscopic picture, however, was similar to but not so pronounced as most of those in the group of chronic nephritics. Here, intercellular fat cells were more numerous in some of the glands, but others showed a definite compactness of structure, vacuolization of cells and pseudoglandular arrangement.

## DISCUSSION

The exact mechanism of the parathyroid hyperplasia in chronic renal insufficiency is still a subject of active debate and no attempt will be made to discuss it here. Phosphate retention, however, is generally admitted to be the initial stimulus and this concept has recently been supported by the production of parathyroid hyperplasia in rabbits by Drake, Albright, and Castleman<sup>15</sup> by the repeated injection of a neutral buffered isotonic solution of sodium phosphate. The hyperplasia so produced is essentially similar to that described in this paper, being characterized by a great increase in the number of chief cells, chief cells, moreover, of normal size and appearance. The only significant difference lies in the lack of oxyphils in the experimental hyperplasias. This may depend on a species difference or may merely be due to the fact that the experiments were not of sufficiently long duration.

Our cases have been presented in four groups: (1) marked chronic renal insufficiency associated with osteitis fibrosa; (2) chronic glomerular nephritis; (3) milder degrees of renal insufficiency; and (4) a group of 9 cases showing parathyroid hyperplasia but without any degree of renal insufficiency. In the first group in which the hyperplasia was most marked and in which bone lesions were also present we have reliable clinical data pointing to a state of severe renal insufficiency of many years duration. In the next group, chronic glomerular nephritis, the duration of the renal insufficiency as judged by the clinical history and by the degree of renal atrophy was also marked but not so great as in the first group. Correspondingly the degree of parathyroid hyperplasia was not so great. The third group presented, showing minor grades of hyperplasia, was made up of a variety of types of renal pathology including nephrosclerosis, polycystic kidneys and pyelonephritis, for the most part secondary to other pathological conditions in the male genito-urinary tract. Although nephrosclerosis and polycystic kidneys represent very long-standing renal disease, functional renal insufficiency is apt to appear only in the terminal stages and to be of comparatively short duration. The fact that all of our cases of chronic glomerular nephritis showed parathyroid hyperplasia appears at first hand quite at variance with Bergstrand<sup>7</sup> who found hyperplasia in only 20 per cent of his nephritics. His paper, however, includes no clinical data and, so far as the negative group is concerned, no classification as regards

types and severity of nephritis from which an estimate of the degree and duration of renal insufficiency can be made.

The studies which we have reported in our previous paper combined with these just presented indicate that histological examination of the parathyroid glands will ordinarily readily permit the distinction between primary and secondary hyperparathyroidism. Primary hyperparathyroidism is the result either of a tumor-like enlargement of one or part of one gland, or of a diffuse hyperplasia of all the glands, sharply characterized by the uniform wasserhelle character of all the cells. Secondary hyperplasia, in contrast, though likewise showing as a rule uniform and sometimes marked enlargement of all the glands, fails to show the same orientation of all the cells toward one line of differentiation. Though chief cells greatly predominate, wasserhelle cells, although much smaller than those seen in primary hyperplasia, are by no means totally suppressed and oxyphil cells are regularly greatly increased in number. With adequate data confusion between these two types of hyperplasia is scarcely possible. However, in view of the inadequate data of many of the cases in the literature, and before we realized the difference in histology and clinical findings, we undoubtedly erred in interpreting some of them.

In our previous paper in the classification of the cases reported in the literature we listed 14 cases of wasserhelle cell hyperplasia and only 2 cases of chief cell hyperplasia. We also listed 10 cases of multiple adenomas of the parathyroid. A review of these 26 cases in light of our further knowledge on the subject calls for a definite reclassification. Probably not more than 5 out of the 14 listed cases of primary hyperplasia really belong to this group. Many of the others belong to the secondary chief cell type, but the majority cannot be accurately classified because of insufficient data. This same criticism might be applied also to some of the 11 cases cited from the literature in the paper by Albright, Bloomberg, Castleman and Churchill<sup>16</sup> in the first report of primary hyperplasia.

A review of the literature, however, suggests the possibility of confusion with the primary adenomas since enlargement of a single gland in association with nephritis has been described on several occasions. Most of these reports are based solely on gross examinations and some of Bergstrand's findings agree with ours — that significant grades of hyperplasia may be present without enlarge-



ment of the gland. It seems fair to assume that in many of these cases histological examination would have revealed significant changes in the other glands.

That a primary parathyroid adenoma should occasionally be found in a patient suffering from chronic nephritis is by no means impossible and it seems probable that MacCallum's <sup>6</sup> case is to be explained in this way. Dr. Fuller Albright has reviewed this case with Dr. MacCallum. They found true osteitis fibrosa and felt that the case was one of a true parathyroid adenoma.<sup>17</sup> Seven of Bergstrand's 10 cases evidently correspond to ours in all essentials. In the remaining 3 enlargement was limited to one gland; the remaining glands were examined histologically and were considered normal. In 1 of these cases, moreover, the abnormality was limited to an adenomatous growth in only a part of the gland. Any doctrinaire opinion of these findings is obviously unwarranted. It is possible that minimal grades of diffuse hyperplasia were overlooked but it is also remotely possible that localized, adenoma-like hyperplasia is occasionally the response of the parathyroid glands in secondary hyperplasia. A tendency in this direction — toward the development of localized, apparently semi-autonomous centers of excessive growth or of peculiarities of differentiation — has been described in our 2 most advanced cases. However, in these instances obvious hyperplasia was evident in the remainder of the gland and also in each of the other glands from the same case, and, moreover, in all of the 38 cases included in this report, not merely those associated with renal insufficiency, hyperplasia when present in one gland was also evident in all the other glands from the same patient. We are, therefore, prejudiced against the occurrence of localized hyperplasia. The study of a much larger amount of material will evidently be necessary to reach a conclusion on this point.

The cause of the gross enlargement of the parathyroid tissue in the various types of hyperparathyroidism has aroused some conflict of opinion. In primary hyperparathyroidism of both the adenomatous and the hyperplastic types a tendency to enlargement of individual cells is very evident. Some authors have felt that this macrocytosis alone without an increase in the number of cells was adequate to explain any grade of enlargement they had seen. As far as the adenomas are concerned it certainly would be difficult to explain a tumor such as that in Case 1 of the Massachusetts General

Hospital series. This tumor weighed 53.2 gm. and although occasional large cells ( $30\mu$ ) are present, the average cell is not over  $15\mu$  in diameter. Macrocytosis may explain a tenfold increase in size but hardly a thousandfold one. Another element tending to increase the gross mass of the glands is the presence in them of dilated acini and cysts containing fluid. In some of the adenomas and most of the primary hyperplasias this must be a significant factor in the weight of the glands. Quantitative studies of the influence of these two factors in relation to the adenomas and especially to the primary hyperplasias are in progress and any opinion would be hazardous without such data.

In the secondary hyperplasias, however, there is no room for argument. The degree of glandular enlargement may run close to a hundredfold, as in 1 of the cases presented, but the predominant cell is a normal sized chief cell and, though larger water-clear and oxyphil cells are present, they are not numerous enough to affect the size of the glands significantly. Moreover, acinar formation is limited and acini where present show small lumens without significant accumulation of fluid. Yet even in these cases where an extraordinary multiplication of cells must have taken place mitotic figures have not been observed.

In our former paper an attempt was made to infer from the cytological evidence presented the probable interrelations and functional significance of the three main types of cells that make up the normal parathyroid. Does the study of secondary hyperplasias carry us any farther in this direction? As regards the interrelation of the cells, it is strongly confirmative of the monophyletic theory. In the normal glands and in the adenomas the fundamental cell was found to be the chief cell and all stages of transitional steps between it and the water-clear cells and the oxyphils were noted. In normal glands such transitional cells must frequently be searched for, but in the active, highly cellular secondary hyperplasia they are extremely numerous, particularly highly vacuolated cells with considerable dense acidophilic cytoplasm which show characteristics of both the water-clear and the oxyphil cells.

The findings in this group of cases appear to fit the hypothesis of a slow progression of development from the chief cell, through the water-clear to the oxyphil. The possibility of direct development of oxyphils from the chief cells cannot be excluded however. We

would suggest that in secondary hyperplasia the rate of this progression is increased, thus accounting for the much greater number of oxyphils than would be expected for the age groups of the patients. The presence of large numbers of oxyphils would on this basis interfere in no way with the theory previously advanced — that they are essentially functionless — since the greatly predominant chief cells would account for the presumable increase in function.

### SUMMARY AND CONCLUSIONS

Another case of "primary" hyperparathyroidism characterized by diffuse hyperplasia of the parathyroid glands of the wasserhelle type is reported. The histological findings in this case have been used to emphasize the contrasting character of the "secondary" hyperplasia which is described in detail on the basis of 29 cases of chronic renal insufficiency of varying grades. Whereas in the primary hyperplasias a uniform direction of differentiation of all cells to the large water-clear type is the invariable finding, in the secondary hyperplasias such uniformity is lacking. Here the glands are composed almost completely of normal sized chief cells, although a few small water-clear cells are occasionally present. The oxyphil cells are always greatly increased in number. The glands show varying degrees of gross enlargement and even when the enlargement is limited to a single gland, microscopic examination has not failed in any instance to show evident hyperplasia in the other glands as well. The criteria for the diagnosis of secondary hyperplasia are described. Comparison of cases of chronic renal insufficiency with and without bone lesions showed quantitative but not qualitative differences in the parathyroid glands, and the development of osteitis fibrosa is felt to be directly dependent on the duration of renal insufficiency. That these changes are in no way specific to renal insufficiency is shown by the fact that no qualitative differences could be recognized between the milder grades of secondary hyperplasia in nephritis and those occasionally seen in individuals without renal insufficiency, but with a variety of associated lesions varying from metastatic carcinomatosis involving bone to basophilism of the pituitary.

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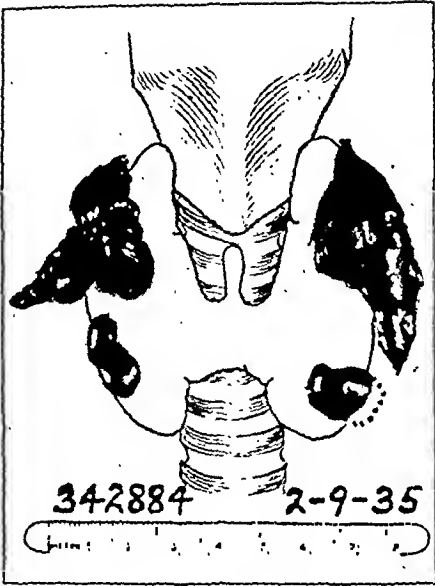
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## DESCRIPTION OF PLATES

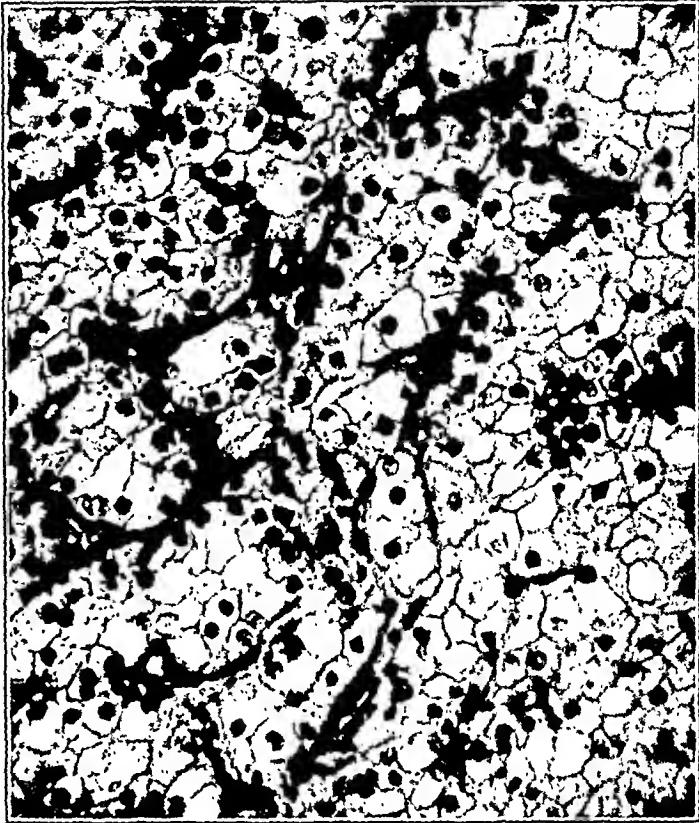
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### PLATE 89

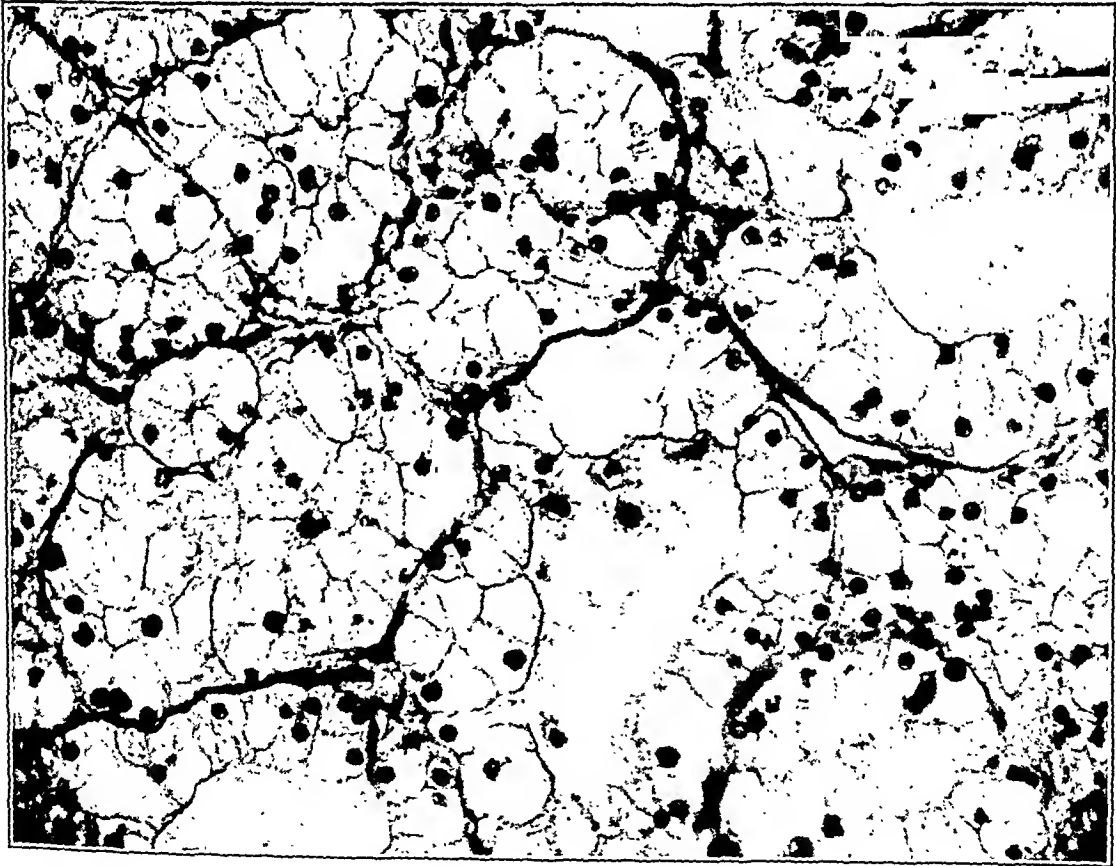
- FIG. 1. A diagrammatic drawing of the parathyroid glands *in situ* from a case of primary hyperplasia. Note the pseudopod-like projection in the upper glands.
- FIG. 2. A microphotograph of a section of one of the glands in Figure 1 showing the basal orientation of the nuclei producing a characteristic pattern.  $\times 500$ .
- FIG. 3. A microphotograph of a section of one of the glands from the same case and at the same magnification. The large wasserhelle cells show a definite tendency to glandular arrangement. Note the resemblance to the hypernephroma cell.  $\times 500$ .



I



2



3

PLATE 90

FIG. 4. A drawing of the parathyroid glands in a case of parathyroid hyperplasia secondary to long-standing chronic renal disease.

FIGS. 5 and 6. A low power microphotograph of a section of one of the glands in Figure 4 showing the circumscribed encapsulated islands of cells.  $\times 10$ .

FIG. 7. A higher power of several of the islands in Figure 5 showing the variation in the type and arrangement of the cells in the different islands. Note the islands of oxyphil cells on the left.  $\times 150$ .

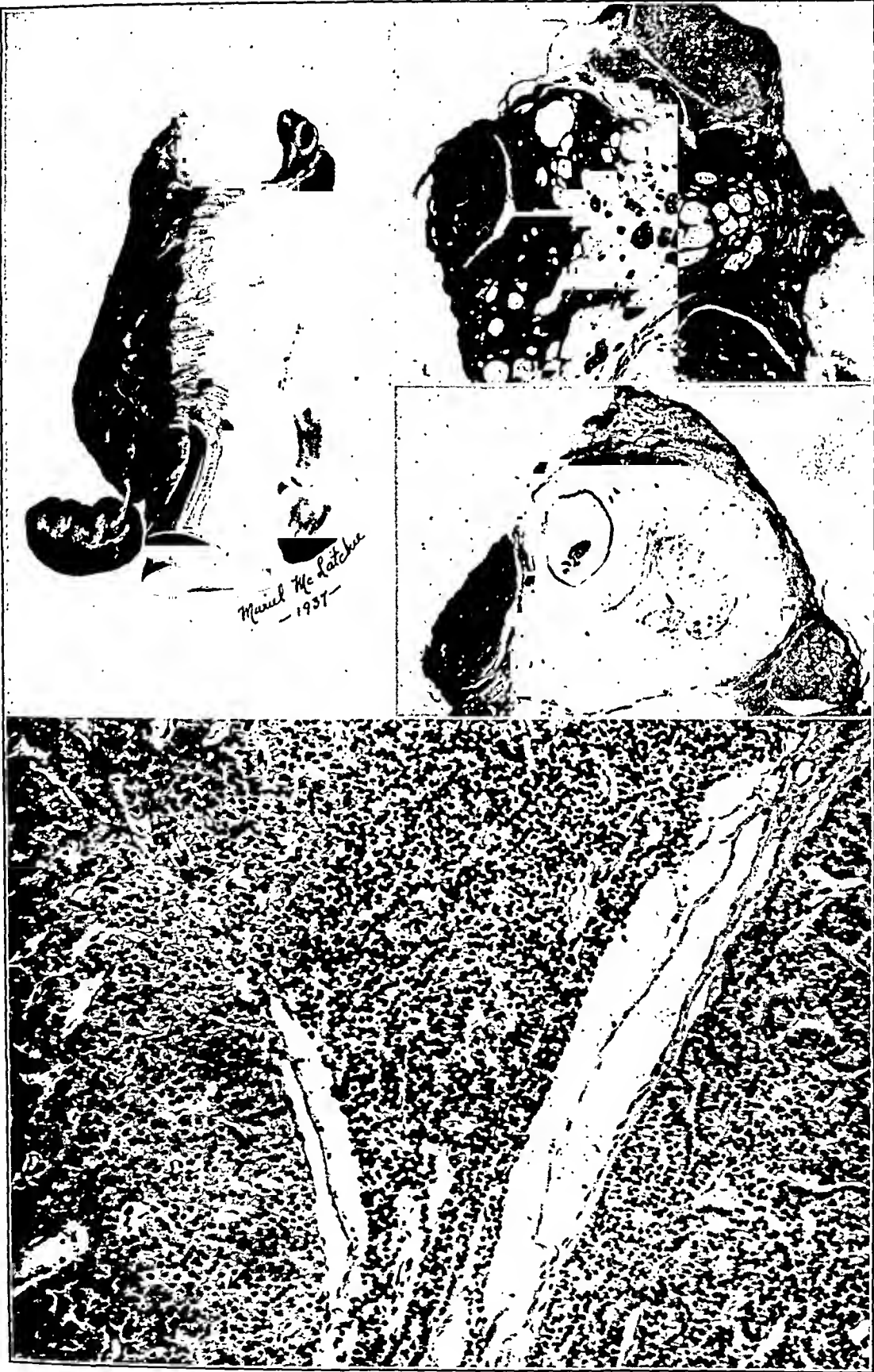
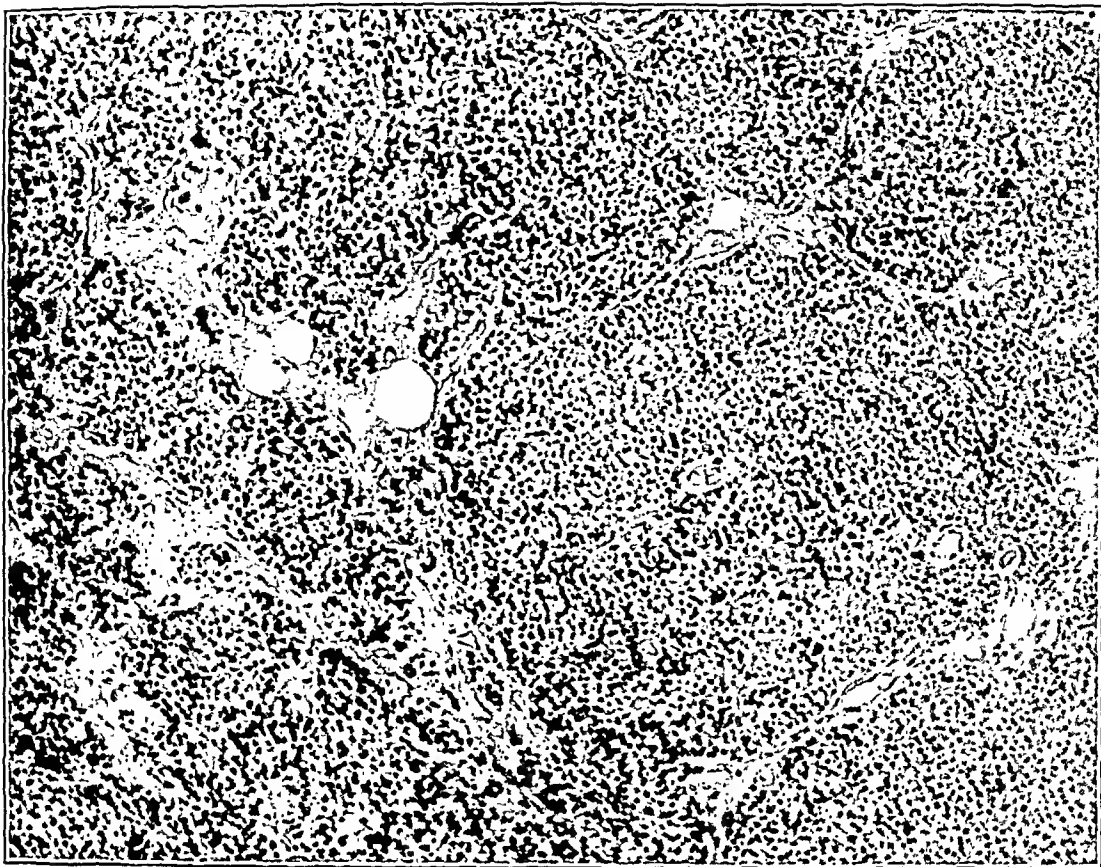




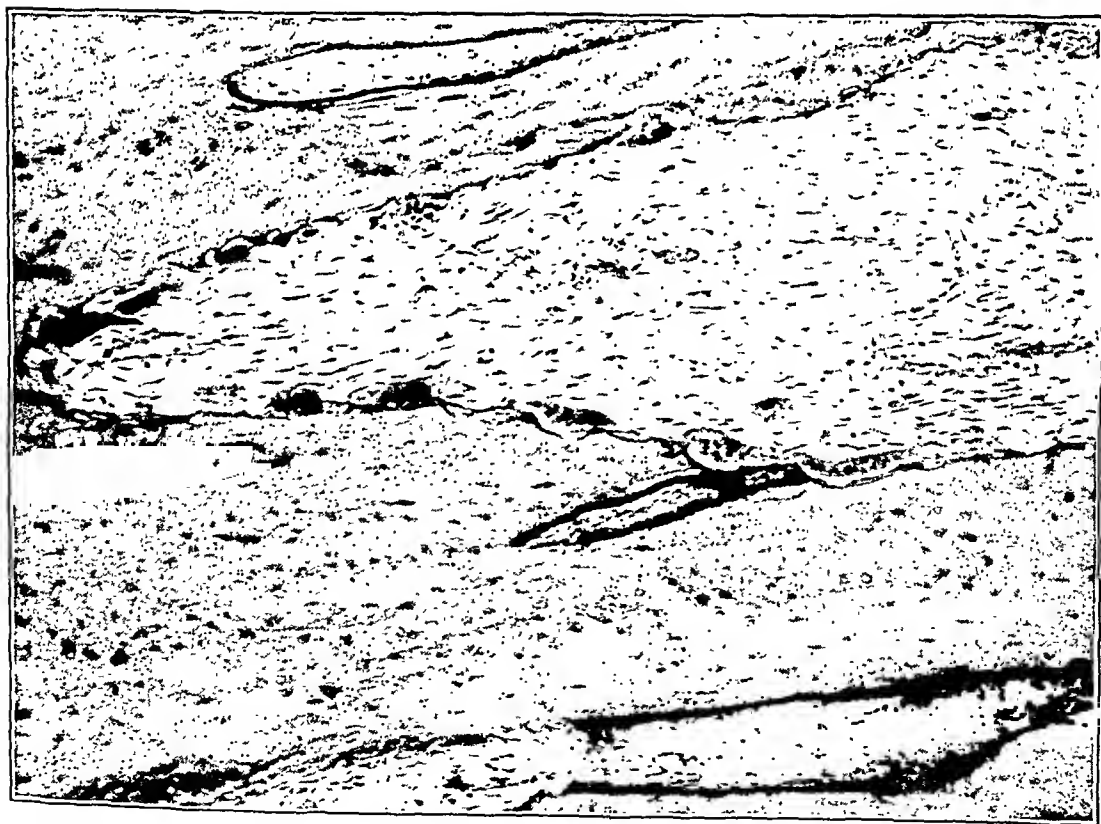
PLATE 91

FIG. 8. A microphotograph of a section of one of the glands seen in Figure 4. Note the cellular compactness, almost complete absence of fat, and the normal sized chief cells. In this gland the nodularity and island formation is relatively inconspicuous.  $\times 150$ .

FIG. 9. A microphotograph of a section of vertebra from the same case showing well marked osteitis fibrosa. Note the large numbers of osteoclasts at the edge of the bone trabecula.  $\times 500$ .



8



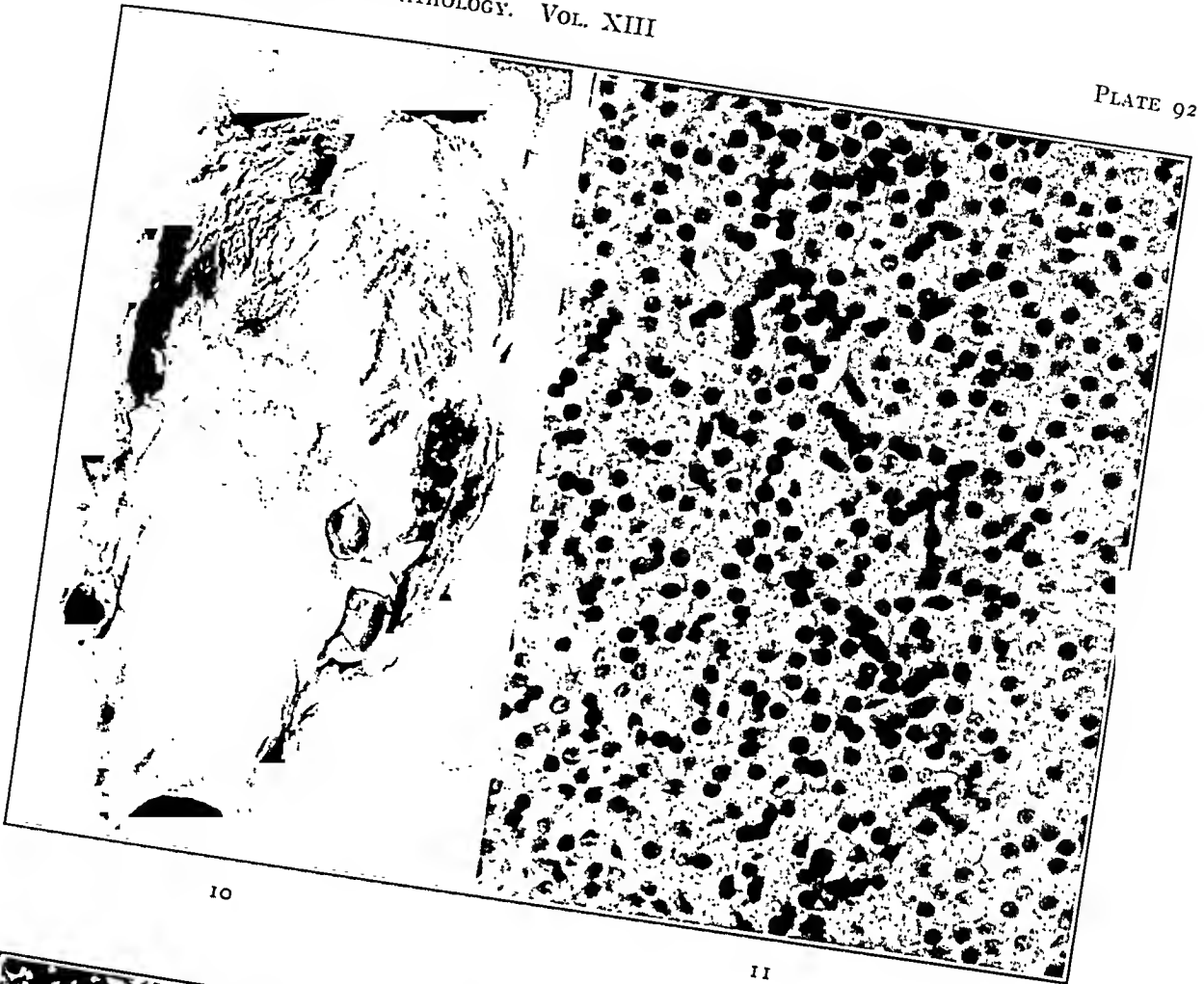
9

PLATE 92

FIG. 10. A photograph of the parathyroid glands in a case of chronic glomerular nephritis. Case No. 10 in Table 1. Note the enlargement and plumpness of the glands.

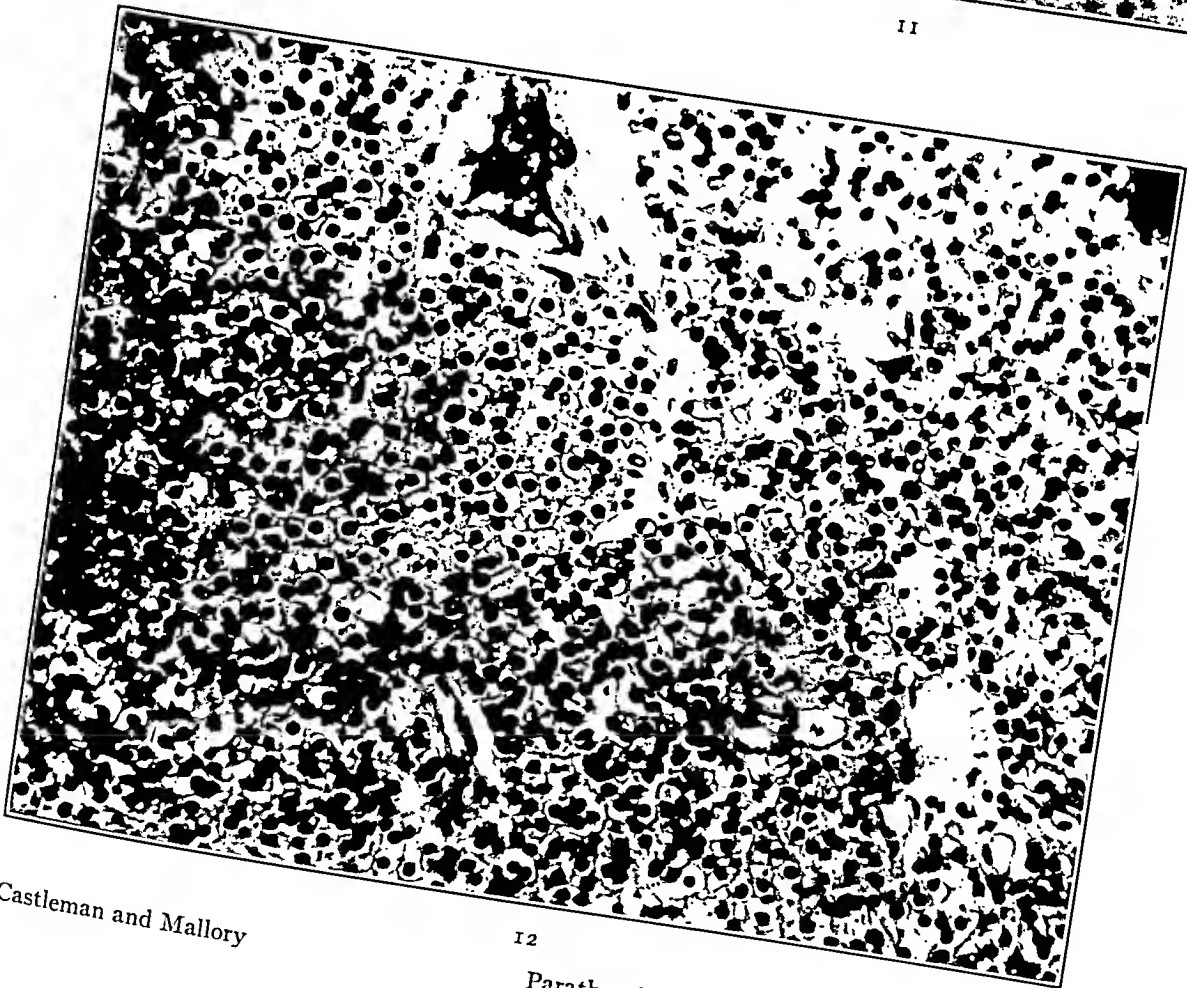
FIG. 11. A microphotograph of a section of one of the parathyroid glands in a case of chronic glomerular nephritis showing the solid sheets of cells without discernible columnar arrangement.  $\times 500$ .

FIG. 12. A microphotograph of a section of one of the parathyroid glands in another case of chronic glomerular nephritis showing the slight vacuolization of the cytoplasm in some areas and the increased number of oxyphil cells.  $\times 500$ .



10

11



12

Castleman and Mallory

Parathyroid Hyperplasia in Renal Insufficiency



## THE RESPONSE OF GUINEA PIG BONE MARROW TO LIVER EXTRACT \*

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Interest in hemopoiesis has received considerable impetus in recent years as the result of the morphological studies of Sabin *et al.*,<sup>26, 27, 28</sup> Doan *et al.*,<sup>6, 8</sup> Jordan and Johnson,<sup>15</sup> Muller,<sup>20</sup> and Peabody.<sup>23, 24</sup> It is generally accepted that very little comprehension of peripheral blood changes obtains without understanding of the basic structural factors underlying these phenomena. Such factors must be sought fundamentally in the histological structure of the bone marrow.

Recently it has been shown by Jacobson<sup>11, 12</sup> that "normal" adult male guinea pigs fed a diet of oats, carrots and lettuce react to the administration of liver extract by the development of a small but significant increase in the number of circulating reticulocytes. Such a response, if constant and consistent, would permit the utilization of this animal as a means of biological assay of the potency of the various liver fractions now in the process of isolation.<sup>16, 18, 31</sup> Hitherto, the only satisfactory means of testing these substances has been their administration to individuals in the relapsed phase of pernicious anemia. The value of utilizing guinea pigs for this procedure is readily appreciated, particularly in view of the absence of any reticulocyte increase, either spontaneously or following the administration of impotent substances.<sup>13</sup> Unfortunately, however, other investigators have questioned the reliability of this test in view of the fact that in their hands guinea pigs showed reticulocyte responses following the administration of materials known to be impotent in pernicious anemia.<sup>1, 10</sup> Definite reticulocyte crises also were said to occur spontaneously.

In this publication the marrow of the guinea pig has been studied under several experimental conditions in order to determine the effect of liver extract and to seek, if possible, the explanation of the disagreement among contemporary investigators. A control series was adequately obtained in a group of stock guinea pigs and no

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attempt was made to utilize other potential reticulocytogenic materials in addition to the liver extract, despite the fact that many such substances are known to exist.<sup>19, 20</sup>

### EXPERIMENTAL

Five groups of guinea pigs were studied. All were composed of adult male animals weighing more than 500 gm. No other general restriction was imposed except for those noted below.

Group I consisted of 6 pigs obtained from the stock used by Jacobson. These animals had been kept under the conditions outlined by him<sup>12</sup> and had subsisted on the prescribed diet for a period of 1 month or longer. They had never received liver extract but were known to have stable peripheral reticulocyte levels.

Group II consisted of 4 pigs obtained from the same stock. These, however, had received liver extract in the past and were known to be consistent, satisfactory reactors. They had received no liver for a month or longer preceding the experiment.

Group III consisted of 7 pigs obtained from the same stock. These animals were also known reactors. They were given by intraperitoneal injection a single test dose of liver extract (commercial extract derived from 4.3 mg. of whole liver per kilogram of pig weight) and daily reticulocyte counts were performed. All 7 showed the expected reticulocyte response (Table I) and were killed on the day following that on which the reticulocyte peak was reached.

Group IV consisted of only 2 pigs of the same stock and was merely included to supplement Group III. An attempt was made here to determine whether or not repeated daily test doses of liver extract (extract derived from 4.3 mg. of whole liver per kilogram of pig weight) would produce an accumulative effect on the bone marrow. Repeated doses were given for 6 and 10 days respectively and the animals were killed at the first indication of subsidence of the reticulocyte response (Table II). Occasionally daily red blood counts were omitted in order to avoid marrow changes resultant on the repeated loss of small amounts of blood.

Group V consisted of 20 pigs obtained from the general laboratory stock. These had not been sustained under the conditions outlined above but had subsisted on a diet of hay, oats and carrots under such conditions as obtained with the modicum of attention and care usually directed toward these animals. Almost all of the animals had

received an injection of suspected tuberculous material  $2\frac{1}{2}$  to  $3\frac{1}{2}$  months preceding the experiment. They were autopsied and examined meticulously for any evidence of disease and all included in this group were normal in appearance. Since all material of the type noted was routinely injected into pairs of guinea pigs, a double check

TABLE I  
*Group III. Reticulocyte Response to Liver Extract*

Guinea pig No.	Liver extract injected	Date of reticulocyte count	Reticulocyte count
509	4/6/36		%
		4/6/36	0.8
		4/7/36	0.8
		4/8/36	1.6
		4/9/36	2.4
		4/10/36	3.0
511	4/6/36	4/11/36	2.2
		4/6/36	1.0
		4/7/36	1.2
		4/8/36	1.4
		4/9/36	2.2
		4/10/36	2.8
523	4/6/36	4/11/36	2.4
		4/6/36	1.3
		4/7/36	1.0
		4/8/36	1.8
		4/9/36	2.0
		4/10/36	2.8
503	5/4/36	4/11/36	2.8
		5/4/36	1.2
		5/5/36	2.2
		5/6/36	3.2
510	5/4/36	5/7/36	3.0
		5/4/36	1.2
		5/5/36	2.4
		5/6/36	3.4
508	5/4/36	5/7/36	3.0
		5/4/36	1.0
		5/5/36	2.0
		5/6/36	3.2
512	5/4/36	5/7/36	2.8
		5/4/36	1.0
		5/5/36	2.0
		5/6/36	3.0
		5/7/36	3.0



was obtained. No pig was used the partner of which showed evidence of disease. The length of time intervening between the injection and sacrifice was considered sufficiently long to obviate attributing any effect to the substance injected.

All of the animals were killed by a sharp blow on the head. Both femora of each pig were immediately disarticulated and dissected

TABLE II

*Group IV. Peripheral Blood Response to Repeated Doses of Liver Extract*

Guinea pig No.	Date	Liver extract injected	Reticulocyte count	Red blood count
			%	million
561	6/29		1.0	
	6/30		1.4	
	7/1		1.4	
	7/2		0.4	
	7/3		1.0	
	7/4	7/4	0.8	
	7/5	7/5	0.8	
	7/6	7/6	1.4	
	7/7	7/7	1.8	
	7/8	7/8	2.2	
	7/9	7/9	2.4	
	7/10		1.8	
555	7/27	7/27	1.2	5.04
	7/28	7/28	1.0	4.54
	7/29	7/29	1.6	4.78
	7/30	7/30	1.8	4.54
	7/31	7/31	1.8	4.52
	8/1	8/1	2.4	—
	8/2	8/2	3.0	5.6
	8/3	8/3	3.0	4.85
	8/4	8/4	4.0	—
	8/5	8/5	2.8	5.76
	8/6		2.0	5.73

free of the attached musculature. The bone was then split and the readily detached pencil of soft, gelatinous marrow was removed. In many of the animals the marrow of the tibia and humerus was also removed and treated in a similar manner. The sternum was occasionally removed, split and immediately placed in a fixative with the marrow intact.

Each pencil of marrow was placed on a dry paper towel and arranged in a concentric spiral fashion so that after fixation a cut section included all levels of the marrow. In many of the specimens

2 minute drops were removed by gentle suction with a capillary pipette. These were transferred to slides previously prepared for supravital staining with a dried film of neutral red and Janus green and studied in the fresh state.<sup>28</sup> As a routine measure, however, imprint preparations were made of all specimens and these were stained by a combination of Wright's and Giemsa's stains.

The marrow was allowed to remain attached to the paper and this was trimmed close to the specimen, after which both marrow and the small segment of paper were dropped into Zenker-formol. The tissue was treated according to the method outlined by Custer,<sup>3</sup> embedded in paraffin, and sectioned as thin as possible. Our preparations averaged about 5 to 6  $\mu$  in thickness.

The supravital stained preparations were used purely for confirmatory histological purposes. Attempts to use them for absolute differential counts were in many cases unsuccessful because of the dense cellularity encountered. Counts of 1000 to 2000 cells were, however, performed on each of the Wright-Giemsa stained preparations. In these, excellent cellular differentiation was obtained within the thinned out periphery of each imprint, but because of irregular distribution the impossibility of identifying the elements in the thick central portion of the imprint, and the imperfect correlation with the evident proportions in the sections, no attempt is made to stress the findings in the present paper.

The observations served, however, to familiarize the author with certain morphological characteristics of the guinea pig marrow constituents and they were used only in a corroborative sense in drawing conclusions.

Critical attention was directed primarily toward the fixed sections and these offered the opportunity for several important observations. Each section was meticulously examined for relative cellularity and the general distribution and arrangement of its components. The changes observed form the basis for this publication. Relatively little difficulty was encountered in identifying the great majority of the constituent elements but the variation in distribution (Fig. 3), the great tendency toward grouping of the less mature elements, and the importance of the mature erythrocytes, accurate counts of which were impossible, precluded the determination of absolute percentage proportions. Additional and applicable data were obtained, however, in the following manner. Each section was ex-

amined with the low power lens to determine general cellularity, fat infiltration, and the presence of bone spicules. With the high dry objective 20 or more different fields were examined in representative portions of the marrow with the purpose of determining again relative cellularity, the number, size and variability of fat vacuoles, and the distribution of megakaryocytes. Oil immersion magnification was utilized to determine the relative proportions and arrangement of immature cells of all types and the same characteristics of eosinophiles, normoblasts, neutrophils and mature erythrocytes.

It is believed that analyses of these elements suffice to establish subsequent conclusions but brief attention will first be directed toward a single element that has occasioned some disputing comment, *i.e.* the megaloblast. Jacobson<sup>11,12</sup> stated that the guinea pig marrow was megaloblastic in character and that the reticulocyte responses to the administration of liver were probably attributable to this fact. Jones,<sup>14</sup> on the other hand, claimed that the elements named by the former author were not megaloblasts but proerythroblasts. It is not my intention to intrude on a long-standing controversial point, but certain facts are quite evident. The marrow of none of the pigs examined was megaloblastic to the extent that such a term implies. There were identified in all of the marrow imprints and supravital preparations, however, cells that have been considered to be megaloblasts (Fig. 4). Similar cells have been regularly observed in fetal liver and pernicious anemia bone marrow imprints but have never been noted personally in normal human bone marrow or in that obtained from individuals suffering from the various types of hypochromic anemia. Tötterman<sup>32</sup> has recently observed these cells in the bone marrow of a patient suffering from hemolytic icterus.

In the Wright-Giemsa preparation the cell measured 14 to 20  $\mu$  in diameter and contained a round or oval shaped, centrally placed nucleus with a scanty rim of deeply basophilic homogeneous cytoplasm. The chromatin was arranged in a fine, fairly regular, shreddy reticular network with a definite parachromatin often exhibiting a vague scroll-like appearance similar to that described by Dameshek.<sup>4</sup> Such a cell answers the description of a megaloblast given by Ferrata<sup>9</sup> and Naegeli.<sup>21\*</sup>

The appearance of the cell contrasts definitely with that of the

\* The identity of this cell as a megaloblast has been confirmed by Dr. Florence R. Sabin in our imprint preparations.

myeloblast (Fig. 4) which possesses a closely packed stippled chromatin with poorly distinguishable parachromatin. Nucleoli are almost always present in the latter cell and vary from 1 to 5 in number. In the megaloblast, however, there are usually none, although occasionally 1 or 2 are seen. Transition stages between this cell and early erythroblasts are often noted. These manifest themselves by a greenish tint to the partially hemoglobinized cytoplasm, an increase of the thickness and diminution of the shreddiness of the chromatin, and an increased prominence and sharpness of outline of the parachromatin.

In the supravital spreads the cell measures between 15 and 25  $\mu$  in diameter and retains the nuclear-cytoplasmic ratio noted above. The cytoplasm has a hazy homogeneous yellowish tint indistinguishable from that noted in the immature cells of the myeloid and lymphoid series but considerably less in intensity than that noted in the fully hemoglobinized late erythroblasts. The color is probably not resultant on any hemoglobin content. A scanty circlet of medium sized coccoid mitochondria usually adheres closely to the nuclear margin and no neutral red staining elements are present.

Whatever this cell may be termed it was present to the extent of 0.1 to 3.5 per cent of the nucleated elements in all of the imprint preparations. A greater prominence is noted in Groups I, II and V. In Group V the values range from 1 to 3.5 per cent, the majority containing more than 2 per cent. In Groups I and II the values range from 0.7 to 1.4 per cent and in Groups III and IV between 1.1 and 0.6 per cent. The significance of these variations will be discussed below.

Examination of the sections revealed several other phenomena that differed from normal human marrow. The increased cellularity of the marrow of the long bones was, of course, one such point. Furthermore, there were relatively enormous numbers of eosinophiles and in certain of the groups megakaryocytes were remarkably abundant. Lymphocytes were consistently present in numbers approaching 15 per cent of the nucleated cells but at no time were follicles observed. In deriving the data noted below, however, the possibility of criticism directed toward inexact morphological distinction in so modified a medium as a fixed and paraffin infiltrated section was considered and only such elements as would permit minimal individual misinterpretation were included.

*Group I:* (Animals fed oats, carrots and lettuce for a period of 1 month or longer without ever having received liver extract.) All of these guinea pigs showed densely cellular marrows with relatively few fat vacuoles and very few bone spicules or areas of fibrosis (Fig. 1). Only two of the specimens showed occasional, small, less cellular areas with increased numbers of vacuoles. In these areas, however, the sinusoids were dilated and contained large numbers of nucleated cells. For the most part the fat was widely scattered and most of the vacuoles were small. They averaged 5 per high power field. All of the sections showed a large number of immature cells, the great majority of which were of the myeloid series. There were many foci in which these cells were sufficiently numerous to constitute the predominant cell in these regions. Mitotic figures were present in remarkably small numbers. Many of the sections showed large numbers of eosinophiles which were present to the number of 35 in the average oil immersion field. Neutrophilic leukocytes were present in large but varying numbers. The less mature band forms were twice as numerous as those containing segmented nuclei. Myelocytes and other immature myeloid cells were present in about an equal proportion in all 5 groups of pigs, but they were more numerous in relation to the mature myeloid cells in only this Group and in Group II. Megakaryocytes were numerous and immature forms of this type of cell were equally as frequent as the mature forms. Occasional high power fields contained as many as 15 of these but the average field in all 6 pigs contained 6. Moderate numbers of normoblasts were diffusely scattered throughout the section but only rarely was any suggestion of island formation discerned. Mature red blood cells were scanty, scattered sparsely, and only occasionally was there any evidence of grouping. So few red blood cells were evident in the sections that only with pointed search were more than occasional erythrocytes noted (Fig. 1a).

*Group II:* (These pigs received the same diet as those described in Group I but had received liver extract in the past and were known to be consistent reactors. They had received no liver for a month or longer preceding the experiment.) The animals exhibited marrows that were indistinguishable from those in Group I. There was dense cellularity and a very small number of vacuoles. Megakaryocytes were abundant and mature red blood cells sparse. Immature cells of the erythroid and myeloid series were slightly increased and

polymorphonuclear cells were abundant. Here also band forms were much more numerous than the segmented. Moderate numbers of normoblasts were diffusely scattered and there was remarkably little grouping of these elements (Fig. 1a). Eosinophiles were conspicuously numerous.

*Group III:* (Animals similar to those in Group II which were sacrificed at the peak of a reticulocyte response to a single test dose of liver extract.) The marrows of these pigs exhibited striking differences from those of the preceding 2 groups. There was moderate variation in some of the sections but on the whole each section showed a definite diminution of cellularity. Vacuoles were enormously increased in size and number and averaged 35 per high power field (Fig. 2). In many areas only scantily filled sinusoids interposed between adjacent fat globules and would have suggested hypoplasia were it not for concomitant changes. Sinusoids barely discernible in Groups I and II were quite obvious in this group. Intersinusoidal capillaries described by Doan,<sup>5</sup> apparently unconnected with sinusoids, contained varying numbers of mature and immature cells. In general the nucleated cellular content of the sinusoids was definitely diminished and the numbers of mature red blood cells considerably increased (Fig. 2a). Even in persistently cellular areas erythrocytes were clearly evident. They were distributed in a diffusely scattered fashion and also appeared as solid cords, as though lying within sinusoids indistinguishable because of the adjacent cellularity. In many areas these erythrocytic cords became widely dilated to form lakes, a feature that was even more noticeable in Groups IV and V. Scattered normoblasts were present in unchanged numbers per unit field but were more manifest as the result of the generally diminished compactness of architecture. Many fields, however, contained large islands of normoblasts aggregated in such a fashion that they were prominently evident, even when viewed with low power objectives (Figs. 2 and 2a), a feature lacking in the initial two groups. Less mature erythroid elements were considerably diminished. Myelocytes and earlier myeloid cells were likewise decreased and were limited to a few, scattered shrunken islands. Mitotic figures were scanty. Megakaryocytes were fewer and were present to the average extent of 1 per high power field. No field contained more than 4 and mature forms of this type of cell predominated. Eosinophiles were likewise less prominent

and numbered about 19 per oil immersion field. Polymorphonuclear leukocytes were slightly diminished but this decrease was relatively greater among the band forms and the ratio of segmented to non-segmented nuclei was only 1:1. More fine bone spicules were present than in Groups I and II, and there was also a slight degree of scattered fibrosis.

*Group IV:* (Given daily test doses of liver extract for 6 and 10 days respectively.) The 2 pigs in this group exhibited some resemblances to all of the preceding 3 groups. There was relatively dense but varied cellularity with only 12 various sized fat vacuoles within the average high power field. Distinguishable sinusoids were distended and filled with cells. These cells, however, were for the most part non-nucleated red blood cells. Throughout the entire section the erythrocytes and normoblasts were numerous, the latter occurring frequently in large islands. Immature cells of both series were likewise present in fairly large groups and mitotic figures were frequent. Eosinophiles were diminished to 16 per oil immersion field and neutrophiles were much increased in numbers. This latter group showed a ratio of segmented to non-segmented forms of less than 1. Megakaryocytes were present in as large numbers as were noted in Groups I and II but all of these were mature in appearance. There were also in the pig killed at the end of 6 days many multinucleated giant cells with vacuolated cytoplasm. The characteristics of these marrows were rather puzzling and for a brief period they remained so until analyses of the next group were completed.

*Group V:* (Animals obtained from the general laboratory stock fed a diet of hay, oats and carrots.) Here, as in the preceding group, there were resemblances to Groups I, II and III. Its distinction in this respect suggested a close relation to Group IV, a presumption that was borne out by the data obtained. The sections showed considerable variability of compactness (Fig. 3). Immediately adjacent to a densely hyperplastic area there often lay one practically devoid of hemopoietic tissue wherein there were large numbers of fat vacuoles. The intervening sinusoids were narrow, compressed and relatively acellular. Such areas occurred as frequently within the diaphysis as they did in the metaphyses, and additional sections cut from deeper levels in the block showed persistence of this arrangement. In general, however, the cellular areas predominated. Bone spicules varied considerably in number and there were rare scattered

foci of fibrosis. Sinusoids were both acellular and cellular in appearance, the latter being packed with nucleated and non-nucleated elements. Immature myeloid and to a lesser degree erythroid cells appeared in large groups, occasionally intermixed, and mitotic figures were focally abundant. Megakaryocytes were present in numbers equal to those in Groups I and II and showed no diminution even in extensively vacuolated regions. Less mature members of this group were again significant. Eosinophiles were abundant throughout and often exhibited narrow strands extending through compressed sinusoids, the sole occupants of these spaces. There were many diffusely distributed polymorphonuclear cells which occasionally encircled compact islands of immature precursors. In this series, again, less mature cells predominated, the ratio of non-segmented to segmented forms being 4:1. Normoblasts were particularly numerous and demonstrated extensive grouping (Fig. 3a). Frequently in hyperplastic segments they appeared as loosely arranged halos about islands of erythroblasts. The non-nucleated red blood cell content was somewhat greater than that of Group III but approximately equivalent to that of Group IV. Here, however, many narrow solid strands of erythrocytes were observed coursing through the denser areas and a few lakes were encountered. Table III demonstrates the contrast of characteristic features in the bone marrows of the various groups.

### DISCUSSION

The initial purpose of this paper was to determine whether the guinea pig showing a peripheral blood response to liver extract would exhibit also a constant change in the bone marrow. It was hoped, too, that the significance of this change, if present, would be established and that in so doing an explanation would be offered for the variance in the results obtained by the different investigators.

Examination of the marrows of Groups I and II, which obviously were entirely similar, demonstrated fundamentally an intensive cellularity with a preponderance of relatively immature forms (Fig. 1a). Early erythroid and myeloid cells were quite prominent and exhibited themselves in large, poorly circumscribed masses. Megakaryocytes, particularly in immature forms, and eosinophilic leukocytes were very numerous. The striking feature, however, was the relative dearth of mature erythrocytes and the absence of well



defined foci wherein evidence of red blood cell maturation was forthcoming. True, normoblasts were present in fairly significant numbers but the numerous large islands visible in the other 3 groups were extremely scanty here.

In view of these and the other observations noted above it is readily conceivable that such marrows have received minimal developmental stimulation resultant presumably on some dietary deficiency. Actual generative powers are unimpaired and the potentiality of immediate maturation or acceleration of preexisting adequate but minimal blood development is obviously the prime characteristic of this marrow.<sup>17</sup> Certainly, the striking changes evinced in Group III demonstrate convincingly that liver extract contains a factor deficient or lacking in the first 2 groups and, further, that its administration produces a definite effect on the histological appearance of the hemopoietic system. Since no other factor entered into the experiment, except for the administration of this substance, such a conclusion is warranted. The guinea pigs of Groups I, II, III and IV were from the same animal room, kept under exactly similar conditions, and were chosen at random from this stock for the several procedures. The only stipulation observed, as has been previously noted, was that those pigs in Groups II, III and IV should be known consistent reactors to liver extract. Seasonal variations noted by Starkenstein<sup>29</sup> were excluded by reason of the fact that animals of all 5 groups were killed at irregular intervals within brief periods of one another. There was no possibility of the introduction of extraneous substances in transit from one laboratory to the other since the pigs were all carried in perfectly clean containers, kept isolated from the general laboratory stock, and killed in less than 2 hours after arrival.

The remarkable diminution in cellularity and replacement by fatty tissue observed in the Group III marrows suggested a fairly pronounced liberation and delivery of blood elements. This has been borne out to a certain extent by the consistent rise in peripheral red blood cell levels noted by Jacobson<sup>12</sup> (see his Table III). The abrupt appearance of large numbers of mature erythrocytes, the numerous islands of normoblasts, the ragged remnants of the islands of hemic precursors, and the marked diminution of megaloblasts created the justifiable impression that some factor had produced a rapid maturation of the cellular marrow and extrusion of the end elements. The

sudden impetus to maturation of erythroid elements evidently had much to do with the lessened numbers of eosinophiles and megakaryocytes noted. These returned to their previous levels in the later groups after acceleration had lessened.

The 2 guinea pigs of Group IV, in themselves, offered insufficient data for conclusive interpretation but coupled with the data obtained from the other groups they served as a definite connecting link. Their status will be considered in connection with that of

TABLE III

*Relative Prominence of Significant Features of Bone Marrow Variation*

Group	I	II	III	IV	V
Immature cells	++++	++++	++	++	++
Megaloblasts	+++	+++	+	+	++++
Mitotic figures	+	+	+	++++	++
Normoblasts	++	++	++++	++++	++++
Erythrocytes	+	+	++++	++++	++++
Megakaryocytes	++++	++++	+	++	++++
Eosinophiles	++++	++++	++	+++	++++
Cellularity	++++	++++	+	++	+++
Fat vacuoles	+	+	++++	++	+++

Group V. The relatively bizarre appearance afforded by the marrows obtained in Group V was at first inexplicable and offered no well defined basis for any conclusion. Areas of relative hypoplasia containing many erythrocytes, normoblasts, and mature granulocytes similar to those in Group III were adjacent to areas of dense cellularity resembling those of Groups I and II. These cellular regions differed, however, in that they contained abundant mitotic figures and both normoblasts and erythrocytes were present in as large numbers as had been observed in the liver response series. Despite apparent focal similarity to Groups I and II this group exhibited no impedance of maturation.

What then was the underlying difference? Groups I and II

possessed marrows filled with immature cells affording them a powerful blood-forming potentiality, the impetus for which was evidently deficient. Adequate maturation for the animals' needs occurred but there remained a large reserve which under the conditions of the experiment was stable. The stimulating factor was obviously contained within the liver extract, for the administration of a small dose of this substance was sufficient to produce a rapid increase of mature elements, depletion of the immature, and readily descried fat replacement. Continued administration of similar doses on sequential days produced in Group IV, not the expected progression of marrow depletion in the long bones, a state that would complete the analogy to pernicious anemia,<sup>24</sup> but a reversion to the type of marrow observed in Group V.

This last group possessed marrows that showed unquestionable evidence of unhampered maturation. Areas of relative hypoplasia were presumed to represent recently depleted storage spaces, whereas those retaining intense cellularity in which, however, maturation was advanced, were considered ripe hemic reservoirs from whence delivery was available in an irregular focal sequence. In much the same manner as the kidney and other secretory units of the living organism exhibit intermittent activity, the hemopoietic system has shown considerable evidence of a similar functional quality. The work of Doan and others<sup>7, 25</sup> gives ample evidence of such cyclic activity.

Definite differences having been observed in the several marrow groups it remained only to determine the factor or factors underlying these variations. The age, stock, sex, weight and environment were essentially the same in all the guinea pigs. The only obvious variation in the experimental conditions was one of diet. Groups I to IV had received oats, carrots and lettuce, with the addition of liver extract in Groups III and IV, and Group V was fed hay, oats and carrots. Investigation of Jacobson's data revealed the fact that in his earlier investigations he had found that animals transported in hay-lined containers and kept in similarly lined bins had been found unsatisfactory for assay purposes because of spontaneous rises of reticulocytes. These animals removed to wire cages and placed on a hay-free diet gradually assumed stable reticulocyte levels and responded satisfactorily to liver extract. Similarly, consistently reacting pigs when permitted to eat hay in addition to their basic diet

rapidly became unstable. No attempt is made to explain the small group of guinea pigs that showed unsatisfactory reticulocyte responses even under the prescribed regimen over a prolonged period. The marrow of none of these animals was studied.

Whether it is the food substance or vitamin content of the hay, the constituents of the molds growing in it, or the element of coprophagy, uncontrolled in Group V, that constitutes the source of the bone marrow stimulating factor is not fully established.<sup>2, 22, 30</sup>

Nor is it definitely known whether any inhibitory factor exists in lettuce. The fact remains, however, that these are the only perceptible conditions in Group V at variance with those in Groups I and II. It is permissible, therefore, in view of the absence of other effective possibilities to conclude that it is the dietary variation that is basic in the production of the hyperplastic, incompletely maturing marrow of the guinea pigs in Groups I and II. The addition of liver extract to the dietary regimen unquestionably produces release from the maturation defect. Continued administration of liver extract permits the marrow to revert to the characteristic unhampered maturation of the uncontrolled pigs in Group V. Cellularity is regained but the proportion of ripened elements is manifestly normal, using as the basis for normality the animals of Group V. The large number of macrophages observed in 1 pig of Group IV very probably was resultant on removal of disintegrating fat occurring in the course of replacement by active tissue. It is also plausible that failure to eliminate completely all sources of reticulocytogenic substances will readily serve to obviate the fundamental purpose of the dietary restriction, the inhibition of hemopoietic maturation.

#### SUMMARY AND CONCLUSIONS

1. Five groups of guinea pigs were studied for the purpose of observing the effect of liver extract on the bone marrow and to determine the mechanism of its action.
2. Ten animals fed a diet of oats, carrots and lettuce showed densely cellular marrow with evidence of depressed maturation of its elements.
3. Seven pigs fed the same diet exhibited marked depletion of cellular content with abruptly accelerated maturation following the administration of a single dose of liver extract.
4. Two pigs fed a similar diet manifested return of cellularity but

persistence of maturation subsequent on the administration of daily doses of liver extract for 6 and 10 days respectively.

5. Twenty pigs from the ordinary laboratory stock fed only hay, oats and carrots possessed a relatively densely cellular marrow which showed no inhibition of maturation.

6. Small and varying numbers of megaloblasts were identified within the marrows of pigs from all 5 groups.

7. The diet of oats, carrots and lettuce obviously lacked a substance available in both liver extract and a diet of hay, oats and carrots. This substance permitted maturation of immature hemic elements.

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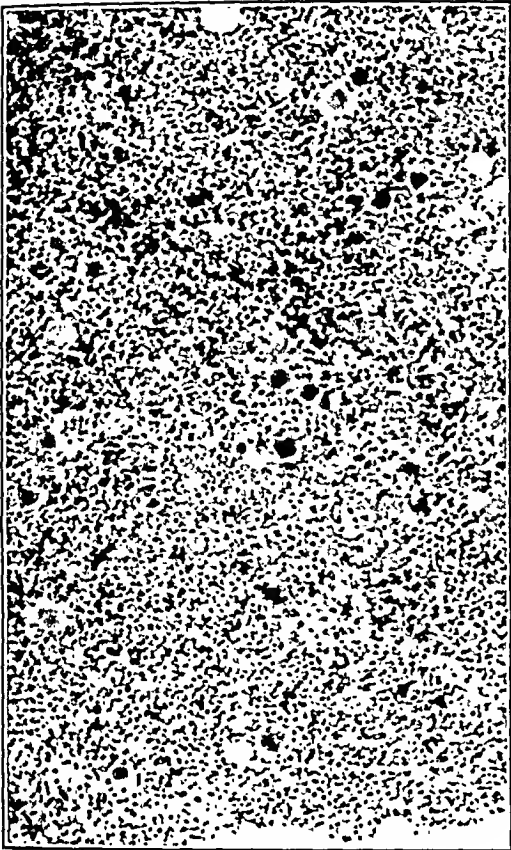
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## DESCRIPTION OF PLATES

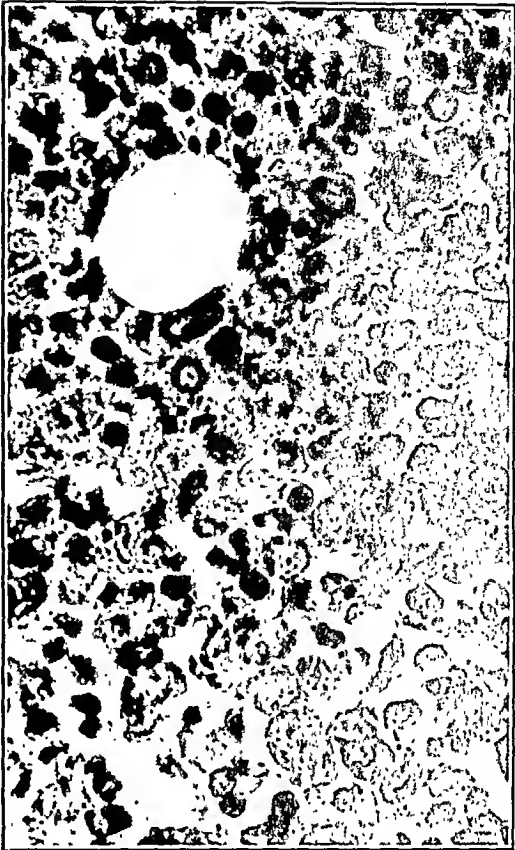
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### PLATE 93

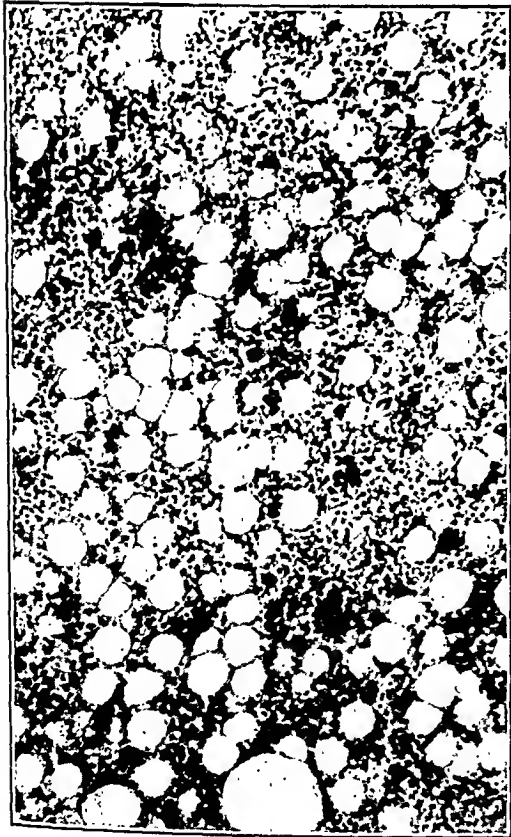
- FIG. 1. Femoral marrow from a guinea pig of Group I. This animal was fed a diet of oats, carrots and lettuce, the prescribed Jacobson regimen. Note the dense cellularity, large numbers of megakaryocytes and scarcity of fat vacuoles.  $\times 100$ .
- FIG. 1a. A similar section at higher power. The large numbers of immature cells, the scantily scattered normoblasts, and the sparseness of mature erythrocytes and granulocytes are evident. Stained with azure II eosin.  $\times 650$ .
- FIG. 2. Femoral marrow from a guinea pig of Group III. This animal was fed a diet of oats, carrots and lettuce. A single test dose of liver extract was given and the pig killed at the peak of the reticulocyte response. There is a pronounced increase in the amount of fat present and many large islands of normoblasts may be observed.  $\times 100$ .
- FIG. 2a. A high power view of a marrow section from a pig of the same group. An island consisting wholly of normoblasts is seen. At its periphery are 2 megakaryocytes and an irregular fringe of mature erythrocytes. Stained with azure II eosin.  $\times 650$ .



I



I a



2



2 a

Gall

Response of Bone Marrow to Liver Extract

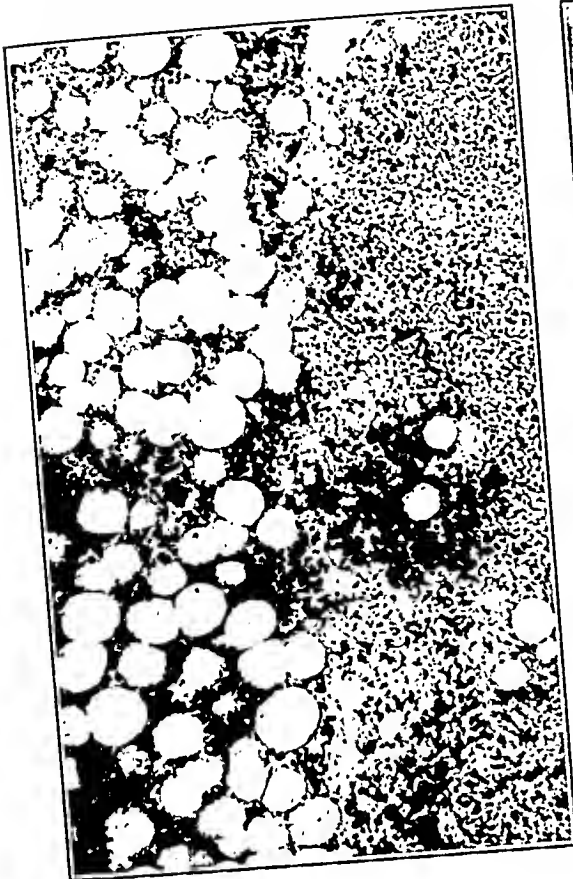


PLATE 94

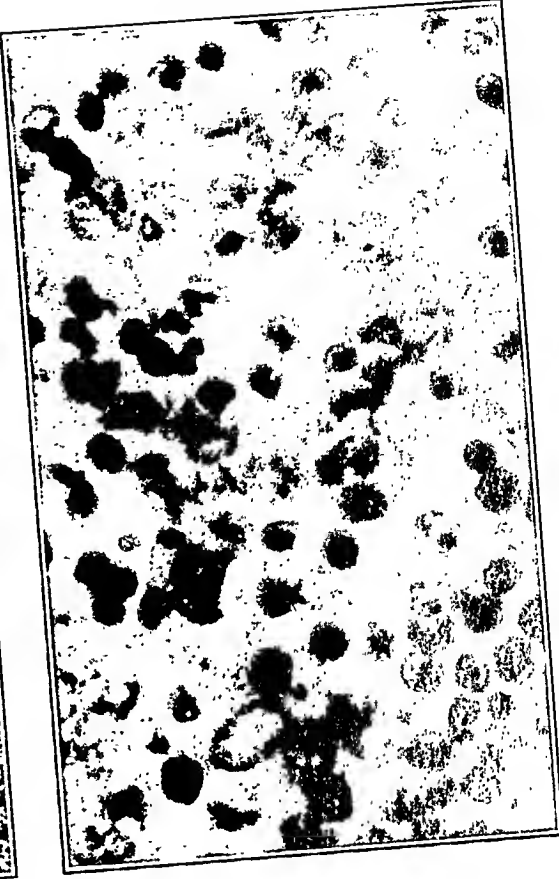
FIG. 3. Femoral marrow from a pig of Group V. This group was fed hay, oats and carrots and was kept in the laboratory stock farm under uncontrolled conditions. The variation in cellularity of adjacent areas is well demonstrated; the cellular area exhibits fairly large numbers of loosely aggregated normoblasts.  $\times 100$ .

FIG. 3a. A high power view of the cellular portion of the same section showing the intermixture of immature and relatively mature cells with no evidence of inhibition of maturation. Stained with azure II eosin.  $\times 650$ .

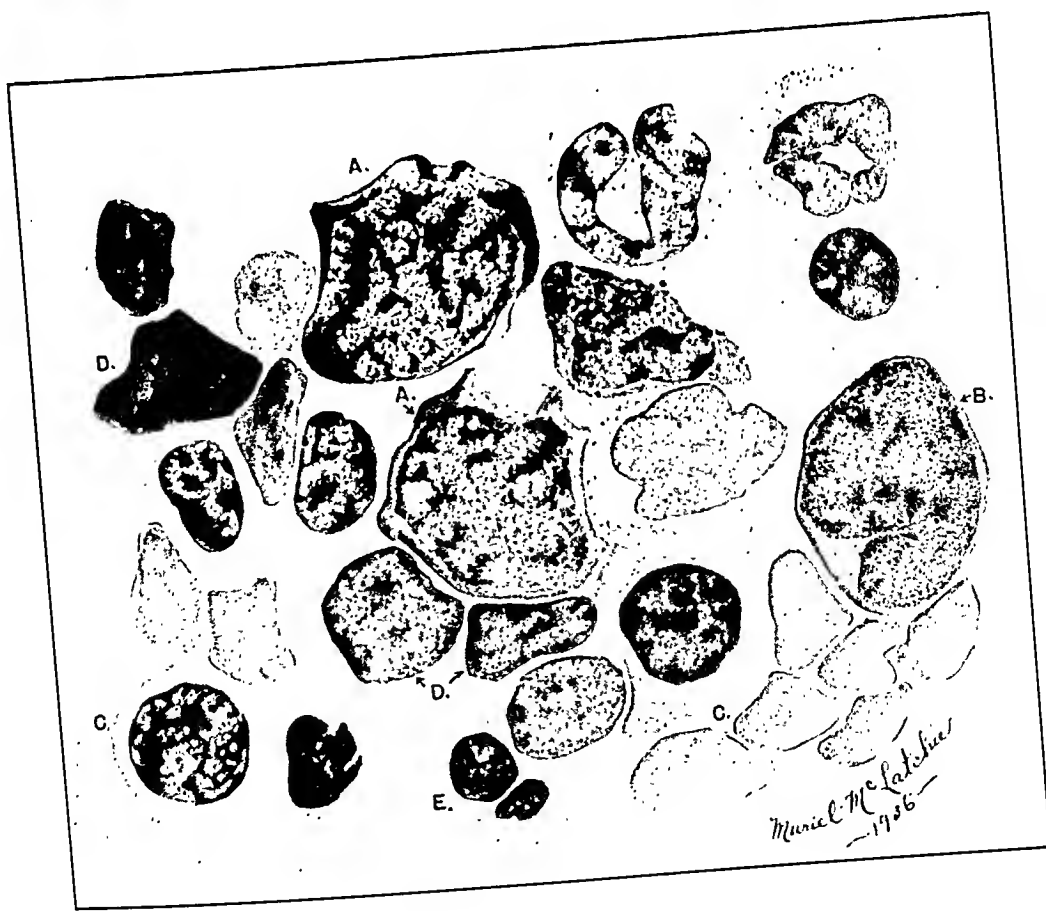
FIG. 4. Drawing of an oil immersion field in an imprint preparation from the femur of a pig in Group V. Two megaloblasts (a) show the shreddy arrangement of the chromatin and in a faint manner the scroll-like parachromatin. The appearance of the nuclei is distinctly different from the stippled arrangement observed in the myeloblast (b). Progressive maturation produces coarsening as represented in the late erythroblast (c) and the lumpy pyknotic chromatin of the normoblast (e). Free nuclei (d) preclude identification. Wright-Giemsa Stain.  $\times 1100$



3



3 a



4

Response of Bone Marrow to Liver Extr

Gall



# THE PATHOGENESIS OF CORTICAL NECROSIS OF THE KIDNEYS IN RABBITS FOLLOWING THE INJECTION OF STAPHYLOCOCCUS TOXIN \*

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There is general agreement that necrosis of the renal cortex is the most constant pathological change occurring in rabbits following the injection of staphylococcus toxin. The mechanism of its production, however, has led to some dispute. Neisser and Wechsberg<sup>1</sup> held that it is an anaemic necrosis secondary to thrombosis of the vessels in this region. VonGlahn and Weld<sup>2</sup> accounted for the tubular necrosis by an interference with glomerular circulation, not so much by thrombosis as by haemorrhage resulting from damage to capillary loops. On the other hand, Rigdon,<sup>3</sup> while describing haemorrhage as a conspicuous feature, believed that the necrosis is the result of a direct action of the toxin on tubular epithelium.

Since necrosis is invariably accompanied by haemorrhage and some degree of thrombosis in the later stages, it becomes necessary to study the earliest changes in order to separate these factors. The present study was directed toward this end by investigating new evidence; namely, early changes in the mitochondria of the tubules.

Previous workers have commented on the wide variation in susceptibility of individual rabbits to staphylococcus toxin. Rigdon, Joyner and Ricketts,<sup>4</sup> for example, found that some animals died immediately after the intravenous injection of 0.5 cc. of their toxin while others received as much as 22.5 cc. over a period of 9 days before death occurred. In the light of recent knowledge concerning the toxin, however, much of this variability can be accounted for and reproducible results obtained. At least two gross errors can be controlled.

First, a normal adult rabbit selected at random may be entirely unsuitable for this type of experiment. Ramon, Richou and Descazeaux<sup>5</sup> have shown that a considerable proportion of normal rabbits contain "natural" antitoxin in their blood serum. This observation

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has been confirmed by Roy<sup>6</sup> in this laboratory for the stock from which the animals in this study were obtained. Therefore, all animals used in this study were selected on a basis of preliminary titrations of their serums.

Second, the potency of the toxins used by previous workers have not been expressed in terms of any defined unit. VonGlahn and Weld<sup>2</sup> have, to some extent, avoided this error by selecting a toxin which, on a basis of previous experience, was known to produce kidney lesions in amounts ranging from 0.1 cc. to 0.5 cc. In the present study toxins were selected that possessed a definite measurable potency in terms of haemolytic activity. The unit employed will be defined subsequently. In a normal rabbit which has no demonstrable "natural" antitoxin, kidney lesions are invariably produced by such a toxin in amounts as low as 0.01 cc.

It is recognized that the measurement of a toxin in terms of its haemolytic content does not necessarily provide information regarding the substance responsible for the kidney lesions. There are at least six well recognized activities of staphylococcus toxin which have been demonstrated repeatedly: haemolytic, leukocytic, dermonecrotic, enterotoxic, plasma coagulating and acute lethal. Burnet<sup>7</sup> and Gengou<sup>8</sup> maintain that a single toxin may exhibit various activities according to the conditions of the experiment. Bigger,<sup>9</sup> Glenny and Stevens,<sup>10</sup> Weld and Gunther<sup>11</sup> and Forssman,<sup>12</sup> present evidence to show that staphylococcus filtrates are mixtures of several kinds of toxins, each fraction being, to some extent, separable from the others. The majority of recent work apparently supports the latter viewpoint. If this be accepted there is no theoretical reason why the measurement of haemolytic activity should predict a content of "nephrotoxic" substance in any given toxin. Practically, however, it has been found in this study that a measurement of haemolysin provides a convenient unit by means of which reproducible results may be obtained provided the animals are selected with a knowledge of the presence of "natural" antitoxin: Again, this "natural" antitoxin is measured in terms of antihaemolytic power.

The present work is not intended to support the theory of the unity of staphylococcus toxins. A personal viewpoint, in fact, favours the conception of separate and distinct fractions and it is probable that the strains of staphylococcus employed were capable

of elaborating several toxic fractions. The measurement of haemolysin does not necessarily imply that it is the substance responsible for the kidney lesions, merely that both haemolysin and kidney necrosing substances are elaborated in amounts showing a sufficiently regular ratio, so that the more easily measured haemolysin is some index of the quantity of the other fraction.

### METHODS AND MATERIALS

*Animals:* Thirty-two normal rabbits weighing from 1300 to 3000 gm. were used. The serum of each was titrated for its anti-haemolytic content previous to the injection of toxin. Twenty-nine of these contained no demonstrable antihæmolytic substance; 3 contained more than enough to neutralize the amount of toxin used.

*Bacteria:* Three strains of *Staphylococcus pyogenes aureus* were used. One was cultured from a human furuncle, a second from a blood culture in a case of fatal staphylococcus pyemia, the third was obtained through the courtesy of Dr. E. O. Jordan as a representative of the enterotoxic strains of staphylococci (Dr. Jordan's No. 5).

*Toxin:* The medium employed was semisolid nutrient agar (0.3 per cent agar) at a pH of 7.2 poured into 15 cm. Petrie dishes in 100 cc. amounts. The staphylococci were heavily seeded from a young agar slant into the semisolid agar which was incubated at 37° C. for 72 hours in an atmosphere of 20 per cent carbon dioxide and 80 per cent oxygen. The whole culture was then centrifuged and the supernatant fluid sterilized by filtration through a Seitz EK filter. This filtrate containing the toxin usually has a pH of about 8.0 and, if sterile, will maintain its hæmolytic power for several weeks with very little loss when stored at 4° C. The filtrate was titrated and used exactly as it was obtained.

*Units:* The unit of toxin employed has been defined by Burnet.<sup>13</sup> It is the least amount of a toxic filtrate that will produce 50 per cent hæmolysis of 1 per cent washed rabbit cells in a volume of 1 cc. in 1 hour at 37° C. This unit has a fairly constant relation to the standard international unit of antitoxin (Hartley and Smith<sup>14</sup>) within an error of 10 to 15 per cent for various batches of toxin. The average lethal dose for normal rabbits whose serum contains no demonstrable antitoxin is about 300 plus or minus 50 Burnet hæmolytic units per kilogram.

*Procedure:* Most of the animals were given a single intravenous injection; a few received several intradermal injections followed by a single intravenous injection 2 days later. Twenty-two rabbits received a sublethal dose (100 to 300 units) of toxin and were killed at intervals afterward. Five received a sublethal dose; the left kidney was removed under sodium amytal anaesthesia after 2 hours and the animal was allowed to live 24 to 48 hours before being killed to remove the right kidney. Two animals were anaesthetized: the upper pole of the left kidney was biopsied, a sublethal dose of toxin was injected intravenously and the left kidney removed after 5 minutes; the upper pole of the right kidney was biopsied after 30 minutes and the animal was killed in 45 minutes. Three animals whose serum contained more than sufficient antitoxin to neutralize a lethal dose of toxin were given a sublethal dose and killed at 30 minutes, 3 hours and 24 hours respectively.

*Autopsies:* A complete autopsy was performed on each animal. This report, however, limits itself to kidney changes.

*Sections:* Duplicate tissues were fixed in Zenker's fluid without acetic acid for general histology, and in Regaud's fluid for the study of mitochondria. Paraffin embedding was employed. The staining methods used routinely on Zenker-fixed material were: haematoxylin and eosin; haematoxylin, acid fuchsin and picric acid; azocarmine, aniline blue and orange G. It was found that sharper differentiation of glomerular basement membranes in the aniline blue technique was obtained by slightly modifying McGregor's <sup>16</sup> formula. Following the azocarmine stain the tissue was mordanted 24 hours in 5 per cent phosphotungstic acid; washed slightly in water and stained 24 hours in aniline blue-orange G. The section, heavily overstained by this procedure, was then differentiated, without washing in water, in dilute alkaline alcohol (1 cc. of 5 per cent sodium hydroxide per 100 cc. of 95 per cent alcohol) for 15 seconds, flooded with dilute acetic alcohol (1 cc. of glacial acetic acid per 100 cc. of 95 per cent alcohol) until the blue returned (about 2 or 3 seconds) then dehydrated, cleared and mounted as usual.

Mitochondria were stained by Masson's <sup>16</sup> method with warmed aniline acid fuchsin and differentiated in dilute picric alcohol after 4 days fixation in Regaud's fluid followed by 6 days in 3 per cent potassium dichromate.

## PATHOLOGICAL FINDINGS

*Gross Changes:* In no instance were gross lesions visible in the kidney within 1 hour after injection, but after 1 hour numerous small, irregularly shaped areas of redness are seen on the surface. The capsule strips cleanly from these areas leaving no bleeding points. On section these areas are seen to be confined to the cortex.

In 2 to 3 hours frank haemorrhage is seen. The surface of the kidney is mottled with irregularly shaped, deep purple areas of haemorrhage ranging in size from pin-point to 2 or 3 mm. The capsule strips readily leaving bleeding points which show that the larger haemorrhagic areas are subcapsular extensions of smaller haemorrhages. On section the haemorrhage is seen to be confined to the cortex and it is most extensive near the capsule.

In 4 or 5 hours larger areas of haemorrhage are seen. They are still confined to the cortex and are most abundant near the capsule. A section at right angles to the long axis of the kidney shows that these haemorrhages are more or less segmentally arranged. That is, regions of haemorrhage extend from the cortico-medullary junction to the capsule in a linear arrangement without interruption and the kidney tissue between appears normal.

After 5 hours the linear distribution of haemorrhage becomes less distinct as the entire cortex is engorged with blood. On stripping the capsule very fine fibrinous adhesions are occasionally broken.

In 12 to 18 hours small yellowish white areas are seen on the surface from which the capsule strips readily. On section these areas are often wedge shaped and invariably are surrounded by a haemorrhagic border.

After 24 hours the white areas extend and tend to coalesce. If the animal survives 7 to 8 days, the maximum damage is seen. Such kidneys exhibit a completely white bloodless cortex over the entire surface. On section the white area is seen extending to the subcortical region where it is sharply demarcated from the medulla by a zone of haemorrhage.

*Microscopic Changes:* In contrast to the gross picture definite changes are seen microscopically within the first 5 minutes. The first change is a dilatation and engorgement of the arterioles and glomerular capillaries. It is not extensive 5 minutes after the injection of the toxin but is undoubtedly different from the normal and



histologically identical with the appearance of such glomeruli at a later stage. The mitochondria of the convoluted tubules in the vicinity of dilated glomeruli show evidence of damage in that many of them become fragmented and tend to assume a spherical shape rather than the normal rod shape. Sections stained by routine methods show no other change in cytoplasm or nuclei at the 5 minute interval.

In from 30 minutes to 1 hour dilated glomeruli become increasingly numerous and the tubular mitochondria are more severely damaged. Definite haemorrhage is uncommon at this stage.

In from 1 to 3 hours mitochondria are reduced to a few globules in the cells of the tubules surrounding dilated glomeruli. Many of these tubules show no mitochondrial substance at all (see Figs. 8 and 9). In other areas of the section where glomeruli are not dilated the tubular mitochondria appear as normal rod shaped filaments. About this time glomerular dilatation appears to be maximal. By following the cortex around the entire extent of a section cut through the whole kidney a pattern of alternating dilated and normal glomeruli may be seen. That is, the dilatation does not appear to be irregularly distributed among the glomeruli but rather in a segmented arrangement. The whole depth of cortex from cortico-medullary junction to capsule in one area shows every glomerulus containing dilated capillaries while immediately beside this region every glomerulus through the entire depth of the cortex appears normal. Thus the whole cortex presents a pattern of alternating segments of dilated or normal glomeruli. Figure 1 is a microphotograph taken at the border between two such segments.

At 3 hours some of the glomerular capillaries are enormously dilated, a single loop may be stretched enough to push the remainder of the tuft into a small area of the glomerular space. Such widely dilated loops are engorged with red cells and stained with haematoxylin-eosin and may easily be mistaken for haemorrhage into Bowman's capsule. With the aniline blue stain, however, the basement membrane of such a loop is seen to be intact although greatly thinned out. Figure 2 illustrates this point. In other glomeruli the basement membrane has ruptured and extravasation of red cells occurred.

Haemorrhage becomes more pronounced during the 3 to 4 hour period. It is most conspicuous in the vicinity of a glomerulus

which itself is engorged with extravasated cells. In many of these glomeruli no remnants of the glomeruli tuft are seen; in others the tuft is reduced to a shrivelled stump at the point of entrance of the afferent vessel while the rest of Bowman's space is filled with red cells (see Fig. 3). The convoluted tubules in this region are either devoid of mitochondria or contain a few globules of various sizes; none is in the form of rod shaped filaments. With routine stains the tubular cells appear swollen and frequently contain hyaline droplets but the nuclei are usually well preserved except in regions where the tubules are definitely necrotic. Fibrin is uncommon but does occur in some of the arterioles and occasionally fine strands are found in haemorrhagic glomeruli.

In from 5 to 8 hours haemorrhage becomes more extensive and necrotic tubules more numerous. In some regions dilated glomerular tufts persist without rupturing and, here, the epithelial cells of Bowman's capsule are altered. Instead of the flat cells with oval nuclei closely applied to the capsular basement membrane normally seen, at this stage one finds cells with swollen cytoplasm bulging into the capsular space and the nuclei are rounded and stain either poorly or very intensely. The latter cells appear to be proliferating. Albuminous deposits are common in the capsular space. In the tuft the perivascular epithelial cells greatly outnumber the endothelial cells and, when the capillary loops are distended, both show some nuclear fragmentation.

In 24 to 48 hours haemorrhage is maximal. It is most conspicuous at the periphery, in the region of ruptured glomeruli (see Fig. 4).

Although necrosis is seen in occasional tubules as early as 5 hours, it does not become extensive until 24 hours. Beginning at the periphery of the cortex and gradually advancing toward the cortico-medullary junction the necrotic zone is always preceded by an area of haemorrhage. Sometimes this haemorrhage outlines a wedge shaped region of necrosis most commonly seen at about 24 hours. After 48 hours these isolated areas tend to coalesce so that eventually the entire depth of cortex is necrotic and a sharp line of haemorrhage separates it from the medulla (see Fig. 5).

Within the necrotic zone the architecture of the glomeruli and tubules is well preserved even when the cells contain no stainable nuclei. Invasion by inflammatory cells is slow although a few polymorphonuclears are seen as early as 24 hours. Even at 5 to 8 days,

when the entire cortex is necrotic, the inflammatory reaction appears to be in its early stages. The invading cells arrive from two regions; from the zone of haemorrhage at the junction of cortex and medulla and from the renal capsule at the periphery, the latter apparently by way of the vessels of the capsule itself. The advancing edge of inflammatory cells always shows pyknotic and fragmenting nuclei as well as cellular debris, the more healthy looking cells being farther back near the blood supply.

### DISCUSSION

The present study shows that the earliest demonstrable change in the kidneys of rabbits receiving an intravenous injection of staphylococcus toxin is a dilatation of blood vessels. This confirms the experience of previous investigators who, however, have not described it as early as 5 minutes after injection. The toxin apparently exerts its first effect on small vessels, as is shown by the dilatation of capillary loops. But the effect must extend farther back in the vascular tree than capillaries, because damage to capillary endothelium alone, while it might result in loss of tone and dilatation of glomerular loops at normal arterial pressure, does not account for the segmental distribution of these dilated glomeruli. In a given segment all of the capillaries are dilated, in another none. If one assumes that arterioles and smaller arteries are primarily involved this segmental pattern becomes reasonably explained. Presumably the small arteries are dilated and all of the glomeruli supplied by them respond to the increased blood flow by dilatation.

The capillaries themselves are also affected by the toxin. The evidence for this is found in the frequent extreme dilatation of a single loop and this is clearly demonstrated in the sections stained by aniline blue (Fig. 2). This stain also provides a means of understanding the mechanism of haemorrhage. Such greatly dilated loops eventually rupture, apparently with explosive force, because another stage is found (Fig. 3) in which the glomerular tuft is reduced to a shrunken residue of frayed ends. Bowman's space is engorged with red cells and red cells fill the lumen of the corresponding tubule.

The toxin exerts a direct effect on the tubular epithelium at an early stage. The evidence for this is found in mitochondrial changes which precede haemorrhage. As early as 5 minutes after the injection of toxin mitochondria begin to lose their structure as rod

shaped filaments, becoming fragmented and globular. This change is more marked in 1 to 3 hours, at which time tubules in the vicinity of dilated glomeruli which have not yet produced haemorrhage often contain no mitochondrial substance at all or merely a few globules (Figs. 8 and 9). At this stage routine haematoxylin and eosin stains usually show no alteration in nuclei or cytoplasm.

Five to 6 hours later the tubular epithelium exhibits changes visible by ordinary stains. The cells are swollen, contain hyaline droplets, nuclei are pyknotic and necrosis is evident. At this time also the epithelium of Bowman's capsule shows evidence of reaction to the toxin by similar changes. Probably this capsular epithelium has been affected at a much earlier stage, since embryologically it is the same type of cell that occurs in the convoluted tubule, but it lacks mitochondria by means of which early changes might be detected.

It might reasonably be expected that if the toxin exerts such damage to renal epithelium in the absence of haemorrhage it must be excreted by the kidneys and therefore be detectable in the urine. This is rarely possible because such small doses are used — 100 to 300 Burnet haemolytic units. Some of the injected toxin no doubt is absorbed by other body cells; some of the amount filtered through the glomeruli is probably concentrated in the tubules and absorbed by them and the final excretion must be so low in toxin that it remains undetectable. Theoretically, much of the toxin is absorbed in the tissue, but even if the total amount injected were excreted in the urine and thereby diluted only 100 times it would not be detectable.

On the other hand, the use of large amounts of toxin defeats this type of experiment. When 10,000 to 50,000 haemolytic units are given intravenously toxin may sometimes be detected in the urine, but the animal dies quickly, usually within 2 or 3 minutes. The problem is open to experiment by the use of formalized toxin and a measurement of the urine's combining power with antitoxin, or by perfusion methods.

The later changes in the kidney leading to large necrotic areas have been adequately described by previous investigators, but one point deserves further comment; the slowness of polymorphonuclear invasion in the necrotic area. Two possibilities suggest themselves: first, the toxin as such may not be so markedly pyogenic as living staphylococci; second, a more reasonable explanation, the toxin is

small in amount, widely absorbed by susceptible cells and not diffusible to a sufficient extent to be pyogenic. From the latter viewpoint the inflammatory reaction may be a response mainly to sterile necrotic tissue.

Controls for these experiments were the animals whose serum contained an excess of antitoxin. By this means comparable amounts of the same batch of toxin were used rather than injections of sterile broth. For example, rabbit No. 440 (Figs. 6 and 7) whose serum contained 640 Burnet antihæmolytic units (approximately 1 international unit of antitoxin) was injected with 260 Burnet hæmolytic units of toxin J5-2 and 3 hours later showed no injury to the kidney. Rabbit No. 437 (Figs. 8 and 9) contained no detectable antitoxin in its serum, was given 300 Burnet hæmolytic units of toxin J5-2, a comparable dose since the average lethal dose is not measurable within a limit of 50 units, and 3 hours later showed marked injury to the kidney. Such results indicate that the injury is due to the toxin and not to other pharmacologically active substances in the medium which might be elaborated during the incubation of the cultures.

### CONCLUSIONS

The sequence of events may be reconstructed as follows: The toxin causes a dilatation of small arteries, arterioles and capillaries (Figs. 1 and 2). It is excreted through the glomeruli and causes direct damage to tubular epithelium (Fig. 9). It injures capillaries, producing loss of tone with subsequent extreme dilatation and eventually sudden rupture (Fig. 3). The sudden bursting of glomerular loops is the mechanism by which hæmorrhage occurs (Fig. 4). Necrosis quickly follows for the tubular epithelium already damaged by the toxin has little resistance to the anaemia resulting from hæmorrhage (Fig. 5). The necrosis is sharply limited to the cortex. Inflammatory cells are slowly mobilized and enter the necrotic area both from the medulla and from the vessels of the renal capsule.

### SUMMARY

1. Attention is called to two errors in previous studies on the effect of staphylococcus toxin on rabbit's kidneys. These are: (a) the failure to select animals on a basis of preliminary titrations of their serums, many of which possess considerable quantities of

"natural" antitoxin; and (b) the failure to measure toxin in terms of some unit of activity.

2. Evidence based on mitochondrial changes in tubular epithelium is presented favouring the view that staphylococcus toxin exerts a direct effect on the kidney cells separate from the changes secondary to haemorrhage.

3. The vascular damage reported previously has been confirmed and the mechanism of haemorrhage described.

4. Cortical necrosis is eventually the result of two factors: (a) direct injury to cells by toxin; and (b) anaemia resulting from haemorrhage.

NOTE: I am greatly indebted to Prof. E. G. D. Murray for his encouragement and criticism throughout this work. I also wish to thank Mr. William Clark for the microphotographs.

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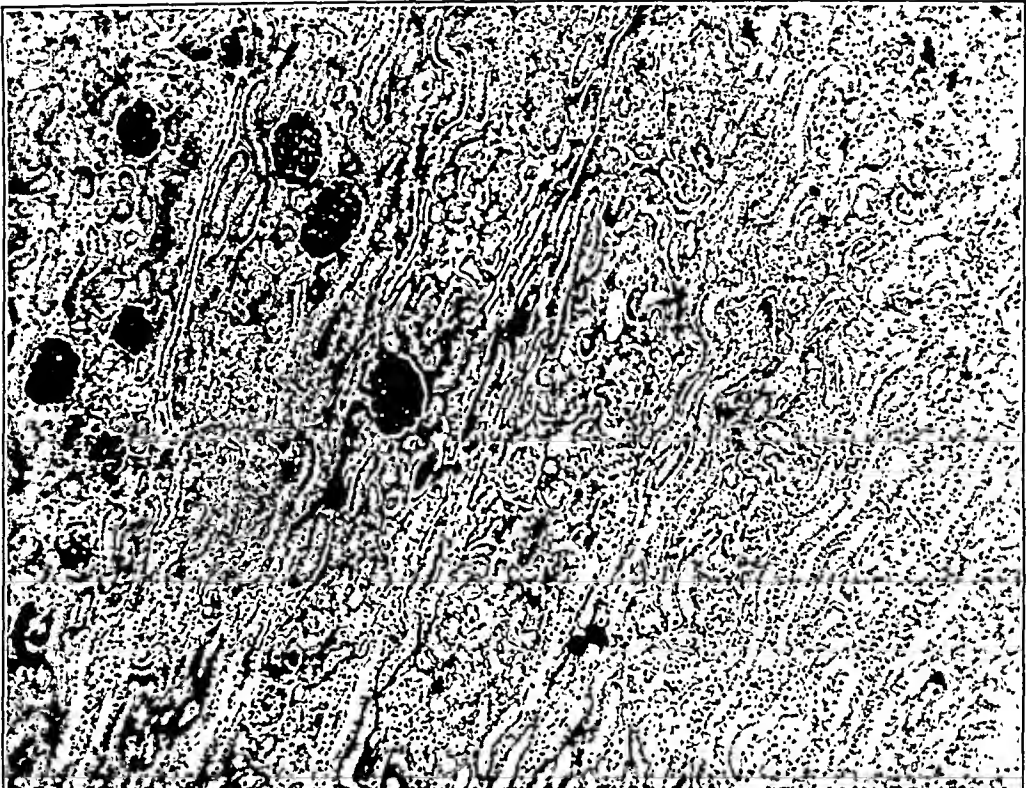
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## DESCRIPTION OF PLATES

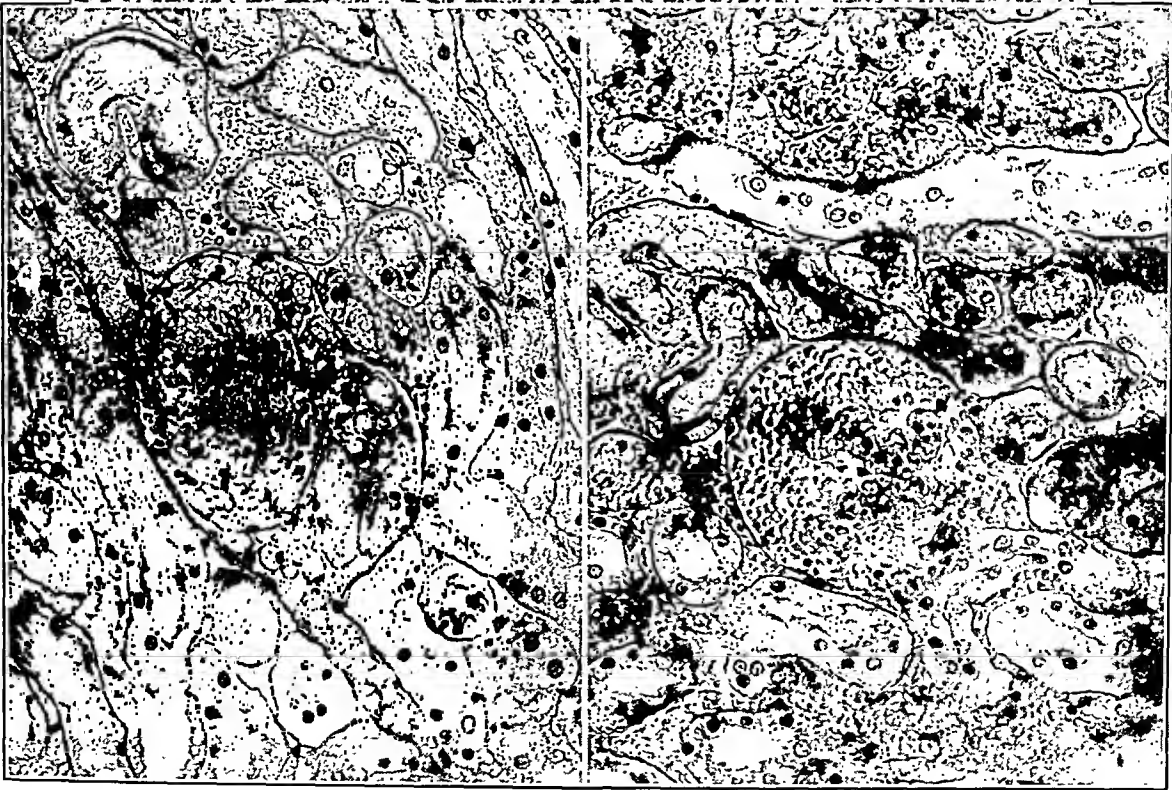
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### PLATE 95

- FIG. 1. The border between two segments of dilated and normal glomeruli 3 hours after the intravenous injection of 300 units of toxin. Haematoxylin-acid fuchsin-picric acid stain.  $\times 70$ .
- FIG. 2. Untouched microphotograph of a widely dilated capillary loop in a glomerulus 3 hours after the intravenous injection of 300 units of toxin. Note that the dilated loop has pushed the rest of the tuft to one side. The basement membrane is thinned out but still intact. Azocarmine-aniline blue-orange G stain. Photographed through Wratten 25 filter.  $\times 270$ .
- FIG. 3. Rupture of a glomerular capillary 3 hours after the injection of 300 units of toxin. Bowman's space is engorged with extravasated red cells and the frayed ends of glomerular basement membrane are barely visible. Azocarmine-aniline blue-orange G stain. Wratten 25 filter.  $\times 270$ .



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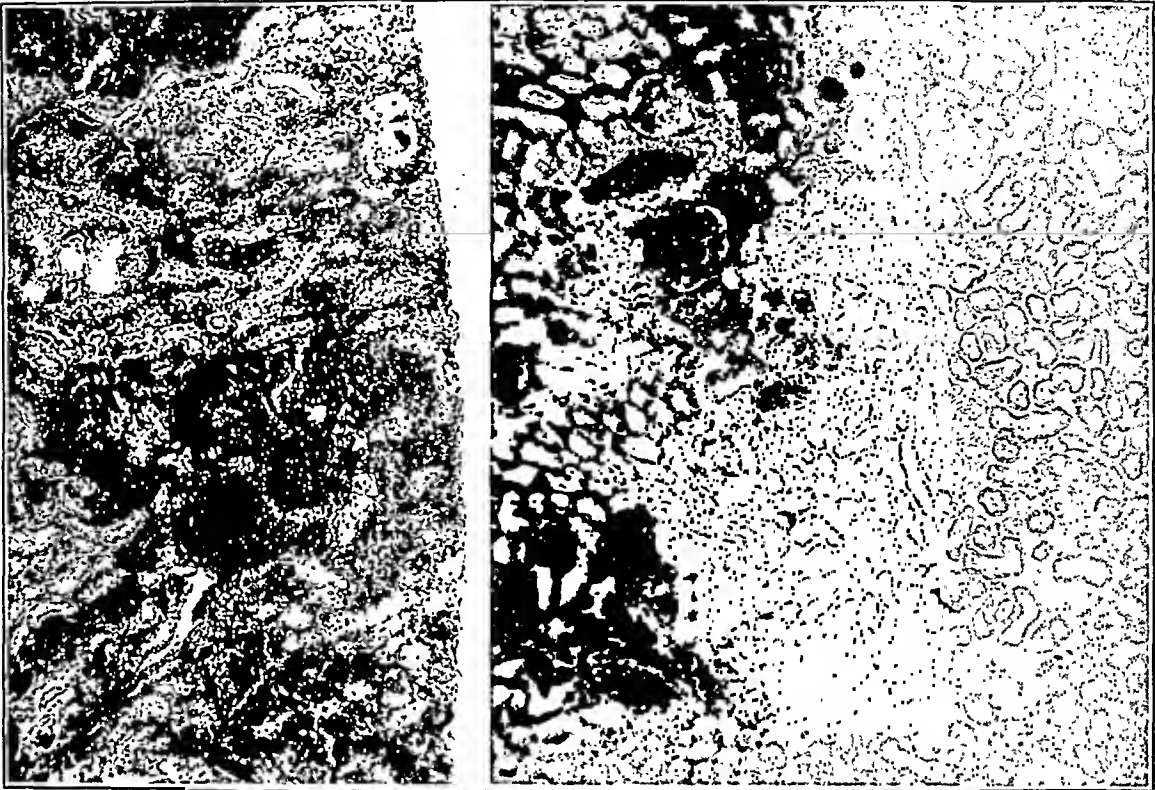
Glynn

Cortical Necrosis of Kidneys

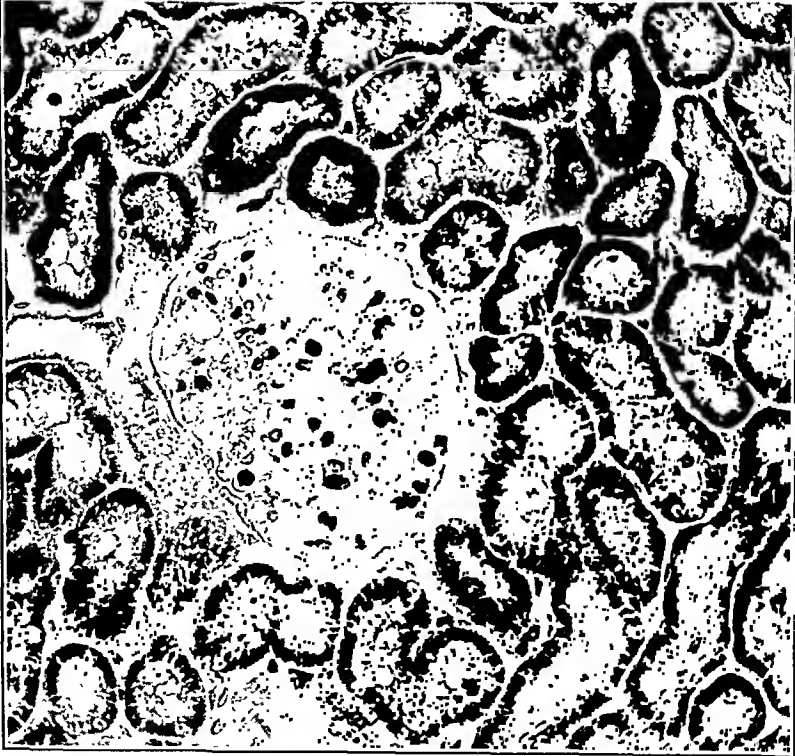


PLATE 96

- FIG. 4. Haemorrhage into the cortex 48 hours after the intravenous injection of 200 units of toxin. The haemorrhage originates in the glomeruli. Haematoxylin-acid fuchsin-picric acid stain.  $\times 70$ .
- FIG. 5. Necrosis of cortex 8 days after the intravenous injection of 150 units of toxin. The cortex is completely bloodless and necrotic. The architecture of the region is preserved although it contains no stainable nuclei. The zone of necrosis is sharply separated from the medulla by an area of haemorrhage. There is beginning invasion by polymorphonuclear cells. Haematoxylin-acid fuchsin-picric acid stain.  $\times 70$ .
- FIG. 6. Mitochondria of tubules 3 hours after the intravenous injection of 260 units of toxin in a rabbit whose serum contained 640 units of antitoxin. The glomerulus is not dilated and the mitochondria of the tubules are normal. Regaud fixation, Masson's acid fuchsin-picric alcohol stain.  $\times 270$ . Compare with Figure 8.



4



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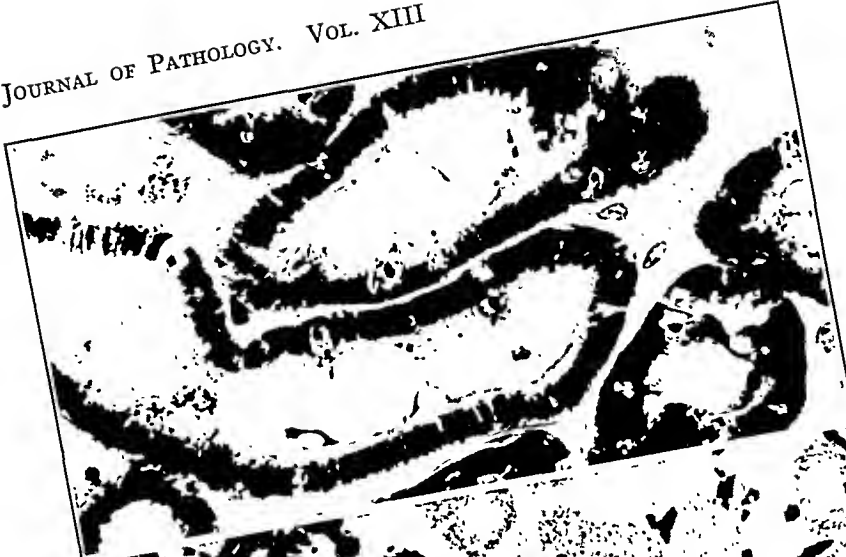
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PLATE 97

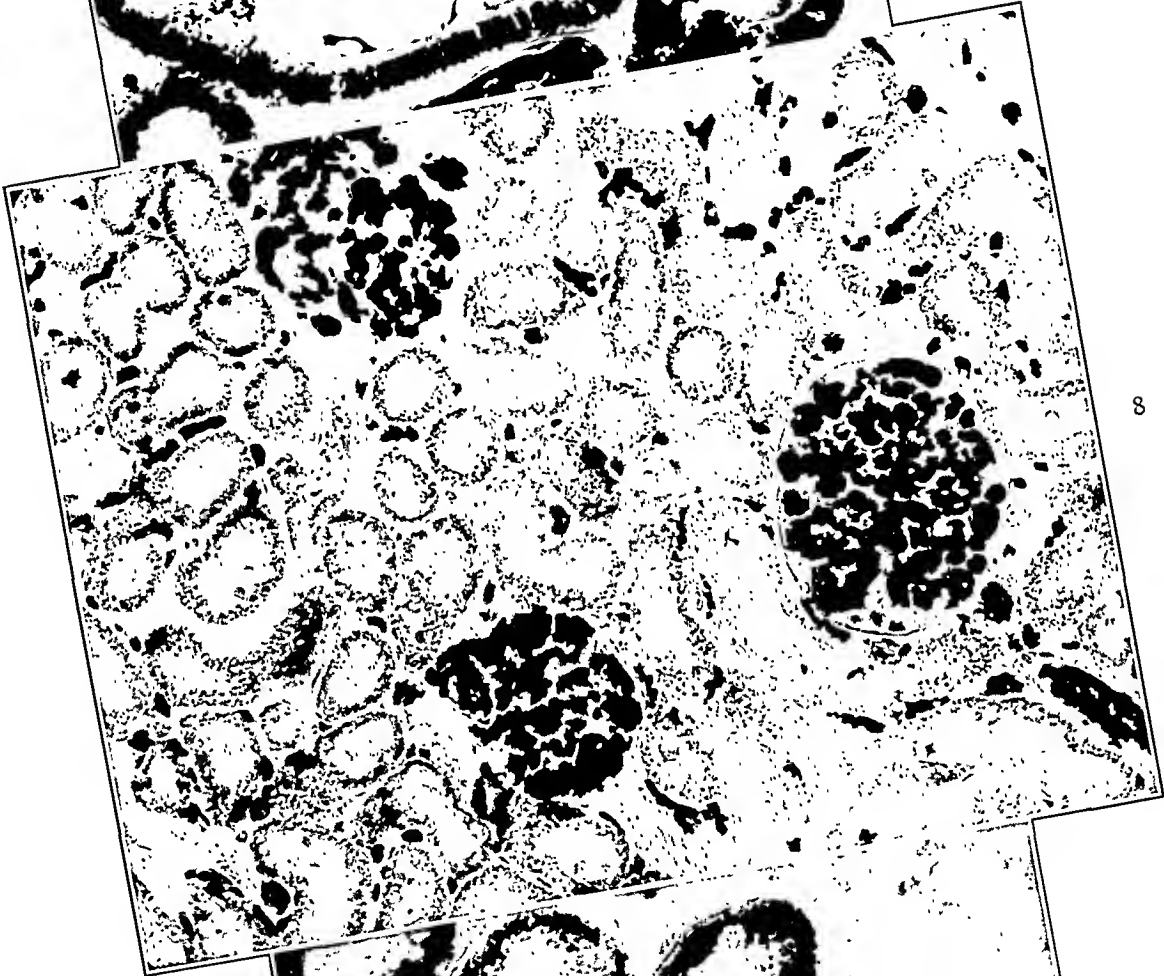
FIG. 7. Same as Figure 6. Detail of mitochondria which appear as normal rod shaped filaments.  $\times 800$ . Compare with Figure 9.

FIG. 8. Mitochondria of tubules 3 hours after the intravenous injection of 300 units of toxin in a rabbit whose serum contained no antitoxin. The glomeruli are dilated and the mitochondria reduced to small globules. Regaud fixation, Masson's acid fuchsin-picric alcohol stain.  $\times 270$ . Compare with Figure 6.

FIG. 9. Same as Figure 8. Detail of mitochondria which are fragmented and globular. A tubule in the lower part of the figure contains no mitochondria.  $\times 800$ . Compare with Figure 7.



7



8



9

Cortical Necrosis of Kidneys

Glynn



SCIENTIFIC PROCEEDINGS OF THE  
THIRTY-SEVENTH ANNUAL MEETING  
OF THE  
AMERICAN ASSOCIATION OF PATHOLOGISTS  
AND BACTERIOLOGISTS  
HELD AT NORTHWESTERN UNIVERSITY,  
CHICAGO, ILLINOIS  
MARCH 25 AND 26, 1937



BUSINESS MEETING  
OF  
THE AMERICAN ASSOCIATION OF PATHOLOGISTS  
AND BACTERIOLOGISTS

Held at Thorne Hall, McKinlock Campus,  
Northwestern University,  
Chicago, Illinois  
March 26, 1937

PRESIDENT FOOT PRESIDING

The Secretary presented the nomination of the Council for officers as follows:

<i>President</i>	ESMOND R. LONG
<i>Vice-President</i>	EARLE B. MCKINLEY
<i>Treasurer</i>	FRANK B. MALLORY
<i>Secretary</i>	HOWARD T. KARSNER
<i>Incoming Member of Council</i>	SAMUEL R. HAYTHORN
<i>Assistant Treasurer</i>	FREDERIC PARKER, JR.
<i>Assistant Secretary</i>	ALAN R. MORITZ

Voted unanimously to elect those nominated.

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Voted to elect the following new members:

Leo Alexander	Harold M. Dixon
Frank C. Andrus	William E. Ehrich
D. Murray Angevine	Harry C. Fortner
Herman Bolker	Edwin S. Gault
G. John Buddingh	David M. Grayzel
John C. Bugher	John C. Grill
Edward L. Burns	Cornelius S. Hagerty
Albert DeGroat	Ernest B. Hanan



Edward H. Hatton	John W. Miller
Louis M. Hellman	Donald A. Nickerson
Sheldon A. Jacobson	Edgar H. Norris
Paul Kimmelstiel	Peter Olafson
Cecil A. Krakower	Antonio Rottino
Richard J. Lebowich	Herbert J. Schattenberg
Amour Liber	Arnold F. Strauss
Louis Lichtenstein	Calvin Torrance

It was also voted to reinstate Drs. H. H. Bullard, J. F. Rinehart and H. M. Zimmerman.

Voted to accept with regret the resignations of Drs. F. B. Gurd, H. Moak, R. Muir, S. T. Orton, F. E. Sondern, R. M. Taylor, J. C. Torrey and M. Wollstein.

Voted to record with deep regret the deaths of Drs. H. M. Adler, B. deVecchi, C. P. Howard, M. Rothschild and F. R. Zeit.

Voted to elect Lt.-Col. George R. Callender as delegate to Congress of American Physicians and Surgeons, and Dr. Ward J. MacNeal as alternate.

The Secretary announced that the next meeting of the Association will be held in Atlantic City, New Jersey, in May, 1938, in conjunction with the Congress of American Physicians and Surgeons.

In discussion of the Symposium for 1938 the Secretary pointed out that because the Congress of American Physicians and Surgeons will utilize the time of one session, the sessions of this Association will be only three in number. Owing to the fact that the number of contributions to the program is increasing each year, the inclusion of a Symposium might be a severe restriction on the number of papers.

Voted to have no Symposium in the 1938 meeting.

The Secretary drew attention to the fact that the Constitution is published with the list of members, and read the following By-Laws which were adopted at the time of organization of the Association.

## BY-LAWS

1. There shall be an annual meeting at such time and place as the Council shall determine.
2. At the first meeting of the Association a Council of Seven shall be chosen, one of whom shall go out of office annually and shall not be immediately eligible for reelection.
3. The Council shall, immediately after its election, determine by lot the terms of office of its members.
4. The vacancy created by the annual retirement of a member of the Council shall be filled by nomination by the Council and election by the Association, and the individual thus elected shall serve for a term of seven years.
5. Should a vacancy occur in the Council, otherwise than by the expiration of the term of service, the Council may elect a member to serve for the unexpired portion of the term.
6. The Annual Dues of the Association shall be ten dollars.
7. The Constitution and By-Laws may be amended by a vote of the Association subsequent to that at which such amendment was proposed, by vote of three-fourths of the members present.

The Secretary then read changes in the Constitution and By-Laws recommended by the Council in order to harmonize current practices with these documents, as follows:

Amend Article II of the *Constitution* to replace the words "Council of Seven, who shall" by the words "Council, which shall."

Replace Article 2 of the *By-Laws* with a new Article 2 to read: "The Council shall consist of seven Members elected by the Association, and the Secretary and Treasurer *ex officio*. The Members shall be elected for terms of seven years each, one Member to be elected annually, and shall not be eligible for immediate reelection."

Delete Article 3.

Change Article 4 to Article 3 and Article 5 to Article 4.

Replace Article 6 with new Article 5, to read: "The annual dues shall be determined by the Council."

Change Article 7 to Article 6.

The Secretary reported that through an oversight the action of the Council last year in reference to the American Board of Pathology had not been made public. Invitations from the American Society of Clinical Pathologists and the Advisory Committee on Medical Specialties had been discussed at two meetings of the Council. During recent years the Council has more and more definitely considered that the function of the Association shall be, as defined in Article 2 of the Constitution, "The advancement of the knowledge of disease," and has been reluctant to embark upon projects that do not directly bear upon this purpose. For this reason the invitations of these two bodies were declined, but at the same time the Council expressed sympathy with the objectives of the American Board of Pathology.

## AMERICAN ASSOCIATION OF PATHOLOGISTS AND BACTERIOLOGISTS

VARIABILITY OF DAILY TUBERCULIN REACTIONS. John Howe (by invitation),  
Chicago, Ill.

*Abstract.* Daily intracutaneous tuberculin reactions, using simultaneous doses of 2 different dilutions of tuberculin PPD, were performed on a series of 4 patients with clinical tuberculosis and 2 control subjects, over periods ranging from 1 to 4 months. The average diameter of the reaction in millimeters was read at 24 hours, and this value was multiplied by the negative logarithm of the dose of tuberculin in millimeters to obtain the Von Gröer product. These products were then plotted on a graph, of which the base-line was the date of injection, to obtain the curves of allergy for each subject studied.

These tuberculin curves show marked fluctuations in the reaction to the same dose of tuberculin, which may be daily or over a period of several days. These fluctuations are present in varying degree in all the subjects studied. The range of fluctuation is from 50 per cent to 100 per cent of the maximal reaction in the patients, and from 55 per cent to 67 per cent in the control subjects.

There is a definite correlation between the state of the peripheral vessels, as measured by daily blood pressure readings under basal conditions, and the fluctuations in the tuberculin reaction. In general, the periods of increased tuberculin reactions are periods of vasodilatation following a period of vasoconstriction, as shown by a falling diastolic pressure following a pressor episode. The correlation between increased tuberculin reactions and falling diastolic pressure ranges from 0.77 to 1.00 in the subjects studied.

### *Discussion*

(Dr. Max B. Lurie, Philadelphia.) I should like to know whether or not the author has found any evidence for desensitization as a result of the repeated administration of tuberculin. In guinea pigs it is frequently seen that if tuberculin is administered at very short intervals desensitization of the animal occurs, and sometimes the reverse happens, especially if the intervals are longer in duration.

(Dr. Howe.) With these small and moderate doses, which I have used, I have found no evidence of desensitization. As you can see from the curves presented, the reactions at the end of the period of observation were comparable with those at the beginning. And neither have I found any evidence for sensitization to tuberculin in these subjects due to the repeated doses.

(Dr. Esmond R. Long, Philadelphia.) I think Dr. Howe has demonstrated beautifully a relationship, a real correlation, with the diastolic pressure. It is interesting that he was not able to make any clear-cut correlation with the clinical course of the disease. Attempts have been made again and again to show such a correlation, but it cannot be worked out. Recently a group of

us followed 116 patients in the wards of the Philadelphia General Hospital over a period of a year with tuberculin tests. We recognized that there were daily variations, and we also considered no variation significant unless it exceeded 50 per cent of the original size of the reaction. We, like Dr. Howe, used 2 tests, a very weak dose and an intermediate one; we did not bother with the standard strong one. But the only correlation we were able to work out was with what might have been desensitization. About 25 pleural effusions developed in the course of the year and this complication usually depressed the intensity of the allergy. Like Dr. Howe, we saw an instance of depression of allergy following sudden pulmonary consolidation due to tuberculosis. It was noteworthy that patients who were improving and patients who were getting worse tended, as the months went by, to fall lower in their intensity of response to tuberculin.

ATTRACTION OF HUMAN POLYMORPHONUCLEAR LEUKOCYTES BY TUBERCLE PROTEIN. William B. Wartman (by invitation), Chicago, Ill.

*Abstract.* In these experiments a study has been made of the chemotactic properties of one of the protein fractions of tuberculin (Seibert's water-soluble tuberculin protein b No. 50). In the undissolved state no attraction for leukocytes could be demonstrated. The protein was then brought into solution and adsorbed on kaolin and on charcoal. In this state strong positive chemotropism was exhibited by the protein in contrast with the weak chemotactic properties of the pure adsorbing agents. By statistical methods it was possible to show that the difference was a significant one. Both kaolin and charcoal were used in order to eliminate the adsorbing agent as a source of attraction.

FURTHER STUDIES ON THE MECHANISM OF IMMUNITY IN TUBERCULOSIS. Max B. Lurie, Philadelphia, Pa.

*Abstract.* A tuberculous rabbit or guinea pig mobilizes the mononuclear phagocytes at the site of inflammation more rapidly than a normal animal, not only in response to reinfection with the tubercle bacillus, but also when a non-specific irritant such as aleuronat is introduced into the pleural cavity. In the rabbit there is no constant correlation between the pH of the exudate and its leukocytic formula, as was also observed by Menkin. There is no evidence of greater seepage of blood proteins into an area of inflammation in a tuberculous as compared with that of a normal rabbit. Nor do antibodies circulating in the blood of rabbits accumulate in the inflamed area to a greater extent in a tuberculous than in that of a normal rabbit.

In the guinea pig there is a definite correlation between the pH of the exudate and its leukocytic formula. The tuberculous guinea pig responds to aleuronat by an exudate which is of lower pH and contains a larger percentage of mononuclear phagocytes than that of a normal animal. Blood proteins and circulating antibodies seep into an area of inflammation of a sensitized guinea pig in higher concentration than in that of a normal animal. Corresponding with these apparent differences in the vulnerability of the blood vessels of tuberculous animals of these two species are differences in their capacity to fix tubercle bacilli of reinfection.

In the rabbit, with large doses, the excessive flow of lymph from the site

of reinfection overcomes the immobilizing factors of the immune animal and accelerates the spread of the bacilli. In the guinea pig, even with large doses of reinfection, the inflammation is of such a character that the flow of lymph is retarded, and the localization of the bacilli is effective.

The extracellular factor inhibiting the growth of tubercle bacilli *in vivo* in the immune animal previously demonstrated by the use of the agar focus cannot be duplicated *in vitro* with the body fluids of the immune animal. Densely woven silk bags were impregnated with a concentration of collodion which prevented the entrance of cells, but admitted the body proteins. If such bags containing tubercle bacilli are placed within the peritoneal cavity of normal animals the bacilli grow unhindered in the bag, while in the tuberculous animal their growth is inhibited. Within these bags the bacilli grow in agglutinated masses in the immune animal; in the normal animal they are more often dispersed. There is no correlation between the thickness of the capsule formed about the bag and the growth of the bacilli within it. Whether this indicates a specific bacteriostatic property of the body fluids of the tuberculous animal or whether this results from physical and chemical conditions which are brought into play differently in the tuberculous than in the normal animal cannot be stated at present. In any event, there is no reason to suppose that these extracellular factors do not play a role in the suppression of growth of tubercle bacilli in the disease itself.

### Discussion

(Dr. Valy Menkin, Boston.) I am very much interested in Dr. Lurie's paper and particularly in regard to his findings in guinea pigs which seem to be similar to what we had found previously in dogs. I was especially interested in his findings in the rabbit, for we had found exactly the same type of response, namely, that there was no strict correlation between the pH of the exudate and the cytological picture. This is contrary to the findings in the dog and in the guinea pig. We have been studying disturbances in the carbohydrate metabolism and the data that are accumulating seem to show that there may be a correlation between the degree of glycolysis and the cytological picture in the rabbit. It is to be remembered that the enzymatic properties of the leukocytes in the rabbit are somewhat different from those in the dog. Dr. Opie pointed that out a number of years ago. The rabbit evidently shows an exceptional type of behavior which ought to be thoroughly studied. It is quite possible that the disturbance in carbohydrate metabolism of that animal may yield intermediary carbohydrate products which may be of importance in determining, at a given time, the predominating type of cell in the rabbit exudate.

(Dr. Paul R. Cannon, Chicago.) I should like to ask Dr. Lurie how he determined the bacterial content in the silk sac, and if he thinks that the reduction in numbers is due to an ablasic antibody, such as Taliaferro has shown for trypanosomes.

(Dr. A. L. Joyner, New York City.) I should like to ask if Dr. Lurie measured the pH of the collodion sac.

(Dr. Lurie.) In regard to Dr. Menkin's remarks, I wish to state that despite the fact that we could not regularly demonstrate pH differences in the exudate of the tuberculous animal as compared with that of the normal animal, there

is much to show that there is a great deal more injury produced on the cells in the exudate of the tuberculous animal as compared with that of the normal animal. Whether this pH inconstancy is accounted for by the neutralizing and buffering effect of the blood proteins I am not ready to say.

In reply to Dr. Cannon, the number of bacilli present in the bag of the tuberculous animal as compared with that of the normal animal was determined by taking a weighed amount of agar from each bag. I did not have a chance to say that the tubercle bacilli were placed inside the bag in molten agar. A weighed amount of the agar inoculum as well as that within the bags was ground as thoroughly as possible; microscopic examination showed the bacilli were completely isolated after grinding. I determined the number of living tubercle bacilli by the number of colonies cultured. Microscopic examination of the agar in these bags showed that fewer colonies developed within the bag placed in the peritoneal cavity of a tuberculous animal, and secondly, and I think that is even more important, in a normal animal the bacilli grow both in colonies and in dispersed form, but in the tuberculous animal they more often grow in an agglutinated manner. Whether this inhibition of growth is due to a specific bacteriostatic property of the serum or body fluids, or whether it is due to a physicochemical change or reaction that takes place differently in a normal as compared with a tuberculous animal I am not ready to say.

The pH of the agar within the silk bag is distinctly lower in the tuberculous animal than in the normal animal, in the small number of observations thus far made. If, as sometimes happens, the growth of the bacilli is significant in the immune animal, then the pH of the agar in that bag is not lower than that in the normal animal.

THE DEVELOPMENT OF TUBERCULOSIS IN NON-ALLERGIC GUINEA PIGS. C. E. Woodruff and H. S. Willis, Northville, Mich.

*Abstract.* In the attempt to throw added light on the question of the relation between allergy and immunity in tuberculosis, the development of that disease has been studied in more than 500 normal, allergic and desensitized guinea pigs. Some groups of animals, rendered allergic by  $R_1$  infection, were desensitized by means of subcutaneous doses of tuberculin gradually increased until 1 cc. of undiluted OT was being given daily. Other groups, infected from the outset with virulent organisms, were kept from developing allergy by means of daily 1 cc. doses of tuberculin. These latter animals fared the worst of any group, showing a 100 per cent mortality, usually by the end of the 3rd month. In all of our mortality studies some of the allergic animals continued alive long after all the desensitized and unsensitized pigs were dead.

Our experiments indicate that the development of tuberculosis is modified to a marked degree in guinea pigs desensitized, or kept non-allergic, by means of large parenteral doses of tuberculin. In these animals the liver and spleen are spared to a large degree, but at the expense of the lungs. There is little or no tubercle formation, the lungs becoming the seat of a fulminating tuberculous pneumonia. Smears from such lungs show innumerable tubercle bacilli. Experimental studies reported in the literature, in which all the animals have been sacrificed before the end of the 3rd month, may fail to give a proper comprehension of this extensive development of pulmonary disease in the desensitized guinea pigs.

PATHOLOGICAL EVIDENCE OF AXONAL AND TRANS-SYNAPTIC PROGRESSION OF VESICULAR STOMATITIS AND EASTERN EQUINE ENCEPHALOMYELITIS VIRUSES. Albert B. Sabin (by invitation) and Peter K. Olitsky, New York City.

*Abstract.* Previous studies on the development with age of localized barriers to the progression of vesicular stomatitis (VS) virus in the peripheral and central nervous systems suggested that the movement of peripherally injected virus occurred in a closed system not only along the axons but probably across the synapses as well. Hurst's studies on Eastern equine encephalomyelitis (EEE) virus led him to state that its movement was not along the axons because it could not spread along peripheral spinal nerves such as the sciatic.

An attempt was made to determine from the distribution of lesions the pathways pursued by the VS and EEE viruses within the CNS, and whether the inability to spread centripetally along certain peripheral nerves implied that axonal progression in general was impossible. Mice were given one or the other virus (1) intracerebrally, (2) intranasally, (3) into the vitreous of 1 eye or (4) into the muscles of 1 hind leg. The whole CNS was sectioned semiserially and the location of lesions noted. After intracerebral injection the lesions were chiefly periventricular, indicating cerebrospinal fluid dissemination, while after the peripheral inoculations the distribution of lesions was wholly different and depended on the central connections of the nerve along which invasion occurred, suggesting primary axonal and trans-synaptic progression. After nasal instillation of either virus in young mice the destruction of neurones in the olfactory pathway from the primary ones in the nasal mucosa through a consecutive chain of 3 to 6 others within the CNS was readily demonstrable. From the vitreous both viruses followed the optic nerve pathway and the constant lesions were (1) in the neurones of the retina, and (2) in those of the contralateral superior colliculus. When the right eye was injected, only the left superior colliculus was necrotic, and only the right one when the left eye was inoculated. The two viruses behaved differently after intramuscular injection. In young mice VS virus passed up the peripheral spinal nerves and produced lesions in the cord. With EEE virus this occurred in only about 5 per cent of the mice, while in the others it was eliminated from the blood onto the olfactory mucosa and the CNS lesions were then the same as after nasal instillation. That EEE virus in the blood invades the CNS by the olfactory pathway was proved by the fact that preliminary treatment of the nasal mucosa with tannic acid, which blocked that pathway, resulted in survival without CNS signs.

THE NATURE AND RATE OF CENTRIPETAL PROGRESSION OF CERTAIN NEUROTROPIC VIRUSES ALONG PERIPHERAL NERVES. Albert B. Sabin (by invitation), New York City.

*Abstract.* The mechanism whereby a neurotropic virus introduced into a muscle invades the CNS along a peripheral nerve, and the relation of alleged differences in the rate of centripetal progression to variations in incubation periods of different viruses were studied. The pantropic pseudorabies and B viruses were employed. (The incubation period after intramuscular injection of rabbits is in the former 50 to 60 hours, in the latter 6 to 7 days.) A constant



amount of virus was injected into leg muscles supplied chiefly by the sciatic nerve and a number of segments of the nerve were tested at varying intervals. By this method no stepwise progression of virus up the nerve could be demonstrated. Instead, with B virus, there was a period of 48 to 72 hours when, with abundant virus in the muscle, none was detectable in any part of the nerve or cord; between 72 and 96 hours the caudal and cephalad halves of the nerve and the cord became positive together and remained positive thereafter. The total amount of virus in the nerve at 96 hours was so small that none could be detected in 1 cm. pieces removed from the middle of either the caudal or cephalad halves, the remainder of which was positive. This type of experiment suggested that primary spread was not by multiplication, rendering the detection of virus at 96 hours possible only in large neural segments. That multiplication occurs later is evident from the increase of virus in the entire nerve, making its detection easy in the small pieces (at onset of paralysis on the 7th day).

The following experiment indicates that intramuscularly injected virus progresses centripetally only along the axon: 1 cm. of the sciatic nerve was excised midway in its course in the thigh; this leaves essentially intact the other neural structures but cuts the axon from its cell body. The excision was made at 48 hours after injection and rabbits were sacrificed at 72 and 96 hours, 8, 9 and 11 days. In none of ten animals was virus detected in the caudal half of the nerve, despite the fact that it was attached to the muscle containing to the end abundant virus, and this result was shown not to be due to the development of inhibitory or antiviral substances in the cut nerve. It was clear that when the axons were severed the spread of virus along them was completely inhibited. At the same time it became evident that no centripetal progression occurred along the other structures of the nerve attached to the muscle.

Studies with pseudorabies virus revealed that none could be detected in the nerve during the first 24 hours; it became demonstrable at 30 to 40 hours in the entire nerve and the cord. It thus appeared that the rate of progression along the nerve did not by itself determine the incubation period. The latter seemed to depend rather on (1) the initial latent period which probably represents the interval before the virus begins to spread along the nerve (B virus 48 to 72 hours; pseudorabies 24 to 30 hours), and (2) the time between invasion of the cord and appearance of sufficient cellular damage to give rise to clinical signs (B virus 3 to 4 days; pseudorabies 12 to 24 hours).

### *Discussion*

(Dr. Arthur W. Wright, Albany.) I should like to ask Dr. Sabin if, while carrying out this work on axonal transmission of viruses, any studies were made of the morphological changes in the affected peripheral nerves. I am wondering whether or not he was able to demonstrate any definite structural changes in the axis cylinders of the nerves that had transmitted virus.

(Dr. Sabin.) At the onset of paralysis, *i.e.*, 3 to 4 days after B virus is first detectable in the peripheral nerve conveying it, widespread lesions are seen in almost all the structures of the nerve, including the axis cylinders. At this time, however, there are also lesions in the neurones of the spinal ganglia and cord, of which the axons are but the protoplasmic extensions. Thus far,

therefore, pathological studies alone have failed to throw light on the course of events. Since the first visible change in the neurone is in the nucleus one must consider the possibility that the virus may have to travel up the long axis cylinders to the nucleus before any increase or multiplication can occur.

**HAPTEN CONJUGATION WITHOUT PROTEIN DENATURIZATION.** Sol Roy Rosenthal, Chicago, Ill.

*Abstract.* While working with phenolphthalein it was noted that this drug administered enterally or parenterally soon appeared in the blood in free and combined forms. For the parenteral route the highest concentration of combined phenolphthalein in the blood was found after 1 to 1½ hours. (Combined phenolphthalein is determined by heating the phenolphthalein serum in a water-bath for 2 hours with concentrated hydrochloric acid and extracting same with ether. The amount of phenolphthalein in the extract is determined in the usual colorimetric way.) The substance to which the phenolphthalein becomes combined is not known, but it is suspected of being protein in nature. This combined hapten-serum was used as an antigen, and injected into a different species of animals than that in which the combination was accomplished. For haptens, phenolphthalein, old tuberculin, acetone soluble and acetone insoluble-methyl alcohol soluble fractions of the tubercle bacillus were used. Precipitin ring tests and skin tests were made, using for antigen the original haptens and the serum of a third species of animal in which the hapten had been injected. Thus, for example, a hapten was first injected into a rabbit intramuscularly; blood was withdrawn after 1 to 1½ hours, and this serum was injected into a guinea pig by any of the parenteral routes. This was repeated every 5 to 6 days for 8 injections. One week after the last injection blood was drawn to be used in the precipitin tests. For antigen use in the precipitin and skin tests the hapten was injected as originally but into a cat or rat, and blood was drawn after 1 to 1½ hours. Control serological and intradermal tests were done using the serum of the same cat or rat before injecting the hapten.

The results indicate that the precipitin tests were not always constant, the best tests being obtained with the extracts of the tubercle bacillus and tuberculin when the combined antigen was injected intravenously. These were positive in dilution up to 1:100,000 of the antigen, and could be completely effaced by adsorption, using the hapten only.

Skin tests gave more constant results, the most striking being with tuberculin, phenolphthalein or the combined serum-hapten antigen. The skin reactions after intracutaneous injection were manifest by induration and redness, at times covering areas 50 by 50 mm. In the case of tuberculin these skin manifestations were already manifest after 1 hour, being most pronounced after 6 to 24 hours, but persisting for 48 to 72 hours, many showing central areas of necrosis. In the case of phenolphthalein the reactions were manifest after 1 to 2 hours and persisted for 48 to 72 hours. Hemorrhages in the lesion were frequent. Control animals yielded little to no reactions. The tuberculin animals after 5 months still give positive skin tests.

This report is a preliminary one. There are many complex features that need solving.

*Discussion*

(Dr. Augustus B. Wadsworth, Albany.) I should like to ask if any precipitation occurs when the acetone-insoluble extract is added to the normal rabbit serum — the rabbit that was not immunized.

(Dr. Rosenthal.) As indicated in the tables projected, normal serum in some instances caused precipitation with the hapten-serum combination, but not as strong as in the immunized animals. No precipitation resulted when the hapten alone was added to normal or immune serum.

SEROLOGICAL STUDIES OF THE REPTILIA. III. STUDIES OF HEMAGGLUTININS IN SNAKE SERUM FOR HUMAN ERYTHROCYTES. Glenn C. Bond (by invitation), Lawrence, Kan.

*Abstract.* In a study of 112 samples of snake serum representing 10 genera and 18 species it was found that 31.2 per cent did not agglutinate human erythrocytes, 19.6 per cent agglutinated types A, B, and AB human erythrocytes, 33 per cent agglutinated all 4 types of human erythrocytes, 1.8 per cent agglutinated A and AB erythrocytes, and 14.4 per cent gave irregular results.

The agglutinins for Types A, B, and AB cells can be specifically absorbed with the homologous cells. The agglutinins for type O cells are absorbed out by any type of human cells. Appropriate snake serum absorbed with human A cells and serum absorbed with human B cells can be used to determine human blood types.

The stability of these agglutinins is comparable to those in human serum.

ISOLATION AND CHEMICAL ANALYSIS OF THE ERYTHROGENIC TOXIN OF *Streptococcus scarlatinae* (NY 5 STRAIN). A. H. Stock (by invitation), Pittsburgh, Pa.

*Abstract.* From a sterile filtrate of NY 5 strain of the hemolytic streptococcus, the toxin was precipitated by two volumes of ethyl alcohol at 0°. The nucleo-protein was removed from the water-soluble part of this precipitate by the addition of acetic acid to pH 4. After neutralization, alcohol precipitation and solution were repeated twice. The precipitate was dialyzed in a No. 600 cellophane bag and then dried. By means of this procedure 0.3 gm. of a viscid substance, drying in thin plates, was obtained from each liter of filtrate. The product is a hygroscopic nitrogenous polysaccharide which contains a minimum of 1.5 per cent ash and 7 per cent nitrogen. It gave no reduction of Benedict's solution, no amino nitrogen with van Slyke procedure, low phosphorus, no halogen or sulphur, negative ninhydrin, faintly positive biuret test, and strongly positive Molisch. After acid hydrolysis, 60 per cent reduction (calculated as glucose) was obtained. The reducing material was N-acetyl glucosamine and galactose. The former was identified by isolation of glucosamine hydrochloride and conversion into the anisaldehyde derivative, and determined quantitatively as 37.5 per cent of N-acetyl glucosamine. Galactose was identified by oxidation to mucic acid. An additional 2 per cent acetyl was present. With the naphthoresorcinol test for uronic acids a dark reddish blue benzol extract was obtained. This extract showed no spectrum. A maximum of 4 to 5 per cent uronic acid was determined by measuring CO<sub>2</sub> from HCl hydro-

lysis. The remaining nitrogen-containing fraction, amounting to 40 per cent of the material, could not be identified owing to interference by glucosamine. One mg. of the toxin (approximately 3 cc. of original broth) gave 75,000 STD. Since 3 cc. of the original broth gave approximately 90,000 STD, it is believed that the bulk of the toxin was removed. The toxin has a proved high immunizing capacity in susceptible individuals.

From Parke Davis peptone, or from the uninoculated medium, a polysaccharide similar in composition to that obtained from the streptococcic filtrate has been isolated. Apparently this fraction is identical with the blood group A substance obtained from pepsin by Schiff and by Landsteiner. Its presence in the Parke Davis peptone (and probably in other peptones as well) is ascribed to the use of pepsin in the manufacture of peptone. Attempts to fraction further the isolated toxin have to date not been successful. Whether the polysaccharide from peptone is an integral part of the precipitated toxin requires further investigation.

### *Discussion*

(Dr. M. L. Menten, Pittsburgh.) Preliminary tests made in our laboratories on a considerable number of peptones showed that all of them gave a very marked Molisch reaction. The question arises as to the relationship of the polysaccharide contained in the uninoculated broth to that of the toxin. In our opinion, one cannot conclude that because the same chemical procedure gives a precipitate from the streptococcic filtrate but fails to give a precipitate from the uninoculated medium, the latter does not contain the constituents of the bacterial polysaccharide. Before one can draw definite conclusions regarding the polysaccharide of the bacterial products found during growth in the culture medium, it is necessary to go back to a critical study of the peptones.

(Dr. Augustus B. Wadsworth, Albany.) Does the antitoxin which is obtained by inoculation of animals with your purified toxin act in the same manner with other toxins as the antitoxin which is produced with your original crude toxin? Streptococcus toxins exhibit peculiar specificities: two toxins may be very closely related but the antitoxin of one will neutralize both, and the antitoxin of the other only the homologous toxin. The antitoxin of the NY 5 strain is broadly valent, whereas some closely related toxins produce antitoxins of much narrower valency.

(Dr. Stock.) We have not used the isolated toxin as yet for immunization to produce antitoxic serums. It has been employed only for immunizing susceptible individuals, and it does immunize them to a negative skin reaction. Our serological tests have been confined to Dick skin tests.

CHEMICAL AND SEROLOGICAL DIFFERENTIATION BETWEEN THE PROTEINS OF DIPHTHERIA TOXIN AND DIPHTHERIA BACILLUS. Monroe D. Eaton (by invitation), St. Louis, Mo.

*Abstract.* The purpose of this work was to determine what proportion of the purified toxic protein, which possesses all of the properties of diphtheria toxin, consists of bacterial protein. The bacterial precipitinogens were detected and measured by precipitin tests with a serum prepared by immunizing a

rabbit with washed diphtheria bacilli. This serum contained very little antitoxin.

The carbohydrate precipitinogens in crude diphtheria toxin are eliminated during the purification of the toxin. One of the bacterial proteins is easily precipitated out of the preparations by one-third saturated ammonium sulphate. A second protein precipitinogen may be partially separated from the toxin by ammonium sulphate and acid fractionation. This bacterial protein, when isolated, gives with the antibacterial serum a precipitin ring at 1:300,000, a dilution of protein 50 to 100 times as great as the corresponding titer dilution of toxic protein against the same serum. Antibacterial serum absorbed with the non-toxic protein precipitinogen gives positive precipitin tests with crude toxin, but not precipitate with the purified toxic protein.

These measurements by the precipitin test indicate that bacterial protein constitutes less than 2 per cent of the total protein in the most highly purified preparations. No protein was present in the culture medium used for production of the toxin. The readily soluble protein, which appears to be diphtheria toxin, is both chemically and serologically distinct from the somatic proteins of the diphtheria bacillus.

A QUANTITATIVE STUDY OF THE RAMON DIPHTHERIA FLOCCULATION REACTION. Alwin M. Pappenheimer, Jr., and Elliott S. Robinson (by invitation), Jamaica Plain, Mass.

*Abstract.* A quantitative study of the diphtheria flocculation reaction by determination of the nitrogen specifically flocculated by the combination of antitoxin and toxin is described. The results of this study have made possible the following calculations and deductions:

1. Diphtheria toxin and antitoxin unite in more than one proportion. The Danysz's phenomenon may be explained in these terms and the magnitude of its effect calculated from the results obtained.
2. The limits of the equivalence zone over which toxin is neutralized by antitoxin have been determined.
3. The amount of nitrogen per Lf unit of diphtheria toxin, the nitrogen per unit of antitoxin and the ratio of antitoxin nitrogen to toxin nitrogen throughout the equivalence zone have been calculated.
4. The analogies and differences between the flocculation and precipitin reactions have been discussed.
5. An absolute method has been furnished for determination of the potency of toxin and antitoxin preparations, even though the strength of neither one is known.

*Discussion*

(Dr. A. M. Pappenheimer, Jr., Boston.) I wish to point out that we have found it necessary to postulate the formation of these soluble intermediates in the union of diphtheria toxin antitoxin in order to explain the Danysz's phenomenon, which is manifest even though no precipitation occurs. The combination of antigen and antibody in multiple proportions completely explains the Danysz's effect only if precipitation occurs, as with the bacterial polysaccharides. Using our results it is possible to calculate and forecast the magnitude of the effect.

TUBULAR DISEASE OF THE KIDNEYS. E. T. Bell, Minneapolis, Minn.

*Abstract.* Tubular disease of the kidneys is a much less frequent cause of uremia than glomerular disease. Eight cases have been studied in which there is satisfactory evidence that uremia was due entirely to injury of the tubules. Six of these were instances of mercuric chloride poisoning, and 2 were due to toxemia. In the latter 2 cases there was a severe hydropic degeneration of all the convoluted tubules.

The symptoms referable to tubular disease are oliguria or anuria and azotemia. Albuminuria and edema are due to glomerular disease.

In the uremia following transfusion with incompatible blood, anuria is due largely to obstruction of the tubules by casts of hemoglobin, but tubular injury may be a contributory factor. In 11 cases of acute uremia with marked hematuria, the renal insufficiency seemed to be due to obstruction of the tubules with blood.

In 8 cases of acute uremia there was marked enlargement of the kidneys and some tubular injury, especially dilatation, but the injury seemed insufficient to have caused uremia and one must suppose that extrarenal influences played a role in causing urinary suppression.

In 7 cases of acute uremia the kidneys were entirely normal microscopically, and urinary suppression must be attributed entirely to extrarenal influences.

The most important factors in the etiology of extrarenal uremia are dehydration and increased destruction of tissue protein. A decrease of blood chloride is apparently not a cause of azotemia.

### *Discussion*

(Dr. Paul Klemperer, New York City.) I should like to ask Dr. Bell about the extrarenal uremia. You have probably seen cases of so-called hepatorenal syndrome which the clinicians are so much interested in, and I wonder what your findings are. In my own experience I could not find any definite change in the kidney to account for the uremia, and I wonder if Dr. Bell's experience has been the same.

(Dr. Virgil H. Moon, Philadelphia.) I am interested in two of the types of renal disease that Dr. Bell has shown: one, the hemorrhagic type, which was associated with septicemia; the other, similar in its hemorrhagic features, in which the condition followed transfusion. I wonder whether Dr. Bell believes that the hemorrhagic features may have originated from a primary injury to the capillary endothelium of the kidney. The reason for making the suggestion is that I have seen lesions similar to these following both clinical shock and experimental shock produced by various means in animals. In those instances the hemorrhagic features were apparently due to direct injury to the capillary walls by whatever agent was responsible for the circulatory deficiency.

(Dr. Bell.) In reply to Dr. Klemperer's question, I have not actually had an example of the hepatorenal syndrome he mentions, but from published reports of these cases I judge they are examples of dehydration, as in intestinal obstruction. This is called hypochloremic uremia, but we know that it is the lack of water and not low blood chloride that causes azotemia.

In the hemorrhagic type of glomerulonephritis which I mentioned I think the injury to the glomerular capillaries is the primary disturbance because

the tubules are usually filled with blood and only occasionally with hemoglobin. But in the transfusion kidney the situation is different. Hemoglobin is formed in the blood stream, passes through the glomeruli and is precipitated in the tubules, obstructing them when the urine is acid.

THE PATHOGENESIS OF CORTICAL NECROSIS OF THE KIDNEY IN RABBITS  
FOLLOWING THE INJECTION OF STAPHYLOCOCCUS TOXIN. John H. Glynn  
(by invitation), Montreal, Canada.

*Abstract.* The wide individual variations in susceptibility of animals to staphylococcus toxins can be controlled by attention to two factors: first, the recognition that a considerable proportion of normal animals possess so-called "natural" antitoxin; and second, the measurement of toxin in terms of some unit of activity. Under such conditions reproducible results are obtained. The present study was directed toward an answer to the question of whether kidney necrosis is due to a direct action of staphylococcus toxin on renal epithelium or secondary to vascular damage. Evidence based on mitochondrial changes preceding hemorrhage supports the former viewpoint, although vascular damage quickly follows and the final picture is believed to be the resultant of both factors. Hemorrhage begins in the glomeruli with extreme dilatation and eventual rupture of capillary loops.

*Discussion*

(Dr. Joseph Tannenberg, Albany.) By chance I made some time ago an observation which greatly supports the view that at least certain poisonous substances injected intravenously may act directly on the epithelium of the convoluted tubules. Rabbits were injected with zinc sulphate intravenously and sacrificed 48 hours later. Within that time the drug in the employed doses produced marked degenerative, necrobiotic changes in the epithelium of the convoluted tubules uniformly throughout the cortex. In one instance, however, a rabbit so injected happened to be afflicted with a spontaneous localized chronic nephritis which had produced many small foci scattered over the cortex. The tubules within these chronically inflamed areas were greatly distended and exhibited a low regenerated epithelium similar to that lining regenerated tubular areas encountered in the course of chronic glomerular nephritis in man (islands of Stoerk). In our case it was striking that these regenerated epithelial cells proved to be entirely free of degenerative changes produced by the zinc sulphate, as if they were specifically protected. This protection was obviously due to the fact that they failed to absorb and concentrate zinc sulphate from the primary glomerular urine, as the normal epithelium of the convoluted tubules did to such a degree that it became necrotic. Under the assumption that the poisonous drug had primarily acted on the blood vessels and by this way influenced the tubules, the protection and preservation of the degenerated tubules would be difficult to explain.

(Dr. A. L. Joyner, New York City.) Some years ago Dr. R. H. Rigdon and I injected toxin in rabbits and reported later on a nephritis we observed, and it was mentioned that we had a good deal of difficulty in getting animals with consistent results, but we observed these early changes in the tubules, and it was entirely on the basis of these that we drew our conclusions. We observed early changes in the mitochondria and cloudy swelling of the tubules at the

same time. We did not lose sight of the fact, however, that when we injected toxin we also got extraordinary changes in the blood vessels, and I disagree with the Doctor that the nephritis is the most common change that takes place in the animal. We saw nephritis only in 25 per cent of the animals. We frequently had sudden death out of some 250 rabbits injected. Sometimes death occurred in 5 to 6 hours, or in 24 hours, and we never saw nephritis. We frequently saw tremendous changes in the blood vessels, and at that time the nephritis mechanism we felt started in the tubules and was a quantitative thing depending on how much toxin was injected and how long the animal survived after the original insult, but we did not lose sight of the fact that the tremendous changes in the blood vessels were the commonest things.

(Dr. Glynn.) In answer to Dr. Joyner, I quite agree with him that if large doses of toxin are used the easiest thing to produce is acute death, and of course if the animals die within a minute, or as early as 5 minutes, there is not much histological damage that can be demonstrated by any staining method I know of. The point I tried to bring out is this — that there are changes that occur in the tubules at an early stage if the animal lives. But the animals must be selected on a basis of preliminary titrations for natural antitoxin and the activity of the toxin must be measured at the time of its use. All these animals were selected on this basis and were given an injection of toxin which was less than a lethal dose. The changes that occur in the tubules can then be shown to be due to the direct action of the toxin and are not secondary to the vascular damage. The hemorrhage is a later phenomenon. These early changes cannot be detected with hematoxylin and eosin stains because a kidney that shows a great deal of damage to the mitochondria by a mitochondrial staining method may by routine methods show no change at all.

EXPERIMENTAL EMBOLIC GLOMERULONEPHRITIS PRODUCED BY HUMAN FAT,  
FATTY ACIDS AND CALCIUM SOAPS. C. S. Hagerty (by invitation), Uni-  
versity, Alabama.

*Abstract.* Lesions similar to those seen in glomerulonephritis in man can be produced in kidneys by the injection of irritants in the form of emulsified human fat, human fat containing soaps, oleic acid and liquid petrolatum into the renal arteries of dogs and rabbits.

Microscopically the fat emulsions injected into the renal arteries affected the kidneys in two ways. The lipins stimulated tissue reactions in the glomeruli, the nature of which depended on the chemical composition of the material injected. The obstruction in the capillary tufts of the glomerulus by the droplets or reactive tissues or both caused atrophy, necrosis, or fatty changes of the cells lining the tubule portion and a subsequent growth of connective tissue about this structure.

The glomerular lesions are of two varieties and depend on the chemical composition of the irritant. Mild irritants cause tissue reactions characterized by endothelial proliferation with a minimum production of collagenous material. Strong irritants produce glomerular reactions in which there is endothelial swelling and marked production of collagenous material. Moderate irritants cause both endothelial proliferation and collagenous production.



*Discussion*

(Dr. E. T. Bell, Minneapolis.) Dr. Hagerty, don't you think that the collagen which you found in the glomeruli is derived from capillary basement membranes? In all the embolic forms of nephritis I have studied the occurrence of collagenous fibers in the glomeruli is due to splitting of the capillary basement membranes. There are no fibroblasts in the glomeruli.

(Dr. Frederic Parker, Jr., Boston.) How did you identify those cells as epithelial cells? In my experience we often see many mononuclear cells, and it is very difficult to tell exactly the type of cell.

(Dr. Hagerty.) In Mollendorf's Handbook of Microscopic Anatomy there is an illustration which indicates that there are fibroblasts in the glomerulus. In their reaction to an irritant these cells might produce collagen. I think that many of the photomicrographs which you saw indicate what Dr. Bell has said, that the fibers seem likely to split off from the basement membrane because they are most numerous in that region. I cannot be certain of the exact nature of the blue staining material which occurs between the endothelial cells.

In answer to Dr. Parker's question, it is a difficult thing to decide whether these cells are monocytes or endothelial cells. However, their appearance and the association of these cells with fibers which lead to a permanent scar indicate to me that the reaction about the fat droplets is a proliferative one.

PARATHYROID HYPERPLASIA IN CHRONIC RENAL INSUFFICIENCY. Benjamin Castleman and Tracy B. Mallory, Boston, Mass.

*Abstract.* Another case of "primary" hyperparathyroidism characterized by diffuse hyperplasia of the parathyroid glands of the wasserhelle type is reported. The histological findings in this case have been used to emphasize the contrasting character of the "secondary" hyperplasia which is described in detail on the basis of 27 cases of chronic renal insufficiency of varying grades. Whereas in the primary hyperplasias a uniform direction of differentiation of all cells to the large water-clear type is the invariable finding, in the secondary hyperplasias such uniformity is lacking. Here the glands are composed almost completely of normal sized chief cells, although a few small water-clear cells are occasionally present. The oxyphil cells are always greatly increased in number. The glands show varying degrees of gross enlargement and even when the enlargement is limited to a single gland, microscopic examination has not failed in any instance to show evident hyperplasia in the other glands as well. The criteria for the diagnosis of secondary hyperplasia are described. Comparison of cases of chronic renal insufficiency with and without bone lesions showed quantitative but not qualitative differences in the parathyroid glands, and the development of osteitis fibrosa is felt to be directly dependent on the duration of renal insufficiency. That these changes are in no way specific to renal insufficiency is shown by the fact that no qualitative differences could be recognized between the milder grades of secondary hyperplasia in nephritis and those occasionally seen in individuals without renal insufficiency, but with a variety of associated lesions varying from metastatic carcinomatosis involving bone to basophilism of the pituitary.

*Discussion*

(Dr. F. A. McJunkin, Chicago.) I should like to ask whether the myocardium was examined for lesions which might be associated with this condition of parathyroid change and with a disturbance of calcium and phosphate metabolism. This perhaps touches only a minor angle of the paper, but I am led to ask the question because in one of a set of experiments on nephrectomized rats it was found that myocardial lesions were present in a certain number of cases. If these bilaterally nephrectomized rats were given small to moderate doses of acid phosphate then the myocardial necroses were present in quite a large percentage of the rats, and from a hurried glance at the tables just now shown it appears that the phosphorus really was quite high in a certain number of the cases. For that reason I should like to ask if acute myocardial lesions were looked for, especially in those cases that had a very high phosphorus.

(Dr. H. Gideon Wells, Chicago.) Cases of renal rickets, so-called, from congenital malformations may produce the most spectacular parathyroid hyperplasias, and in one such case which we saw recently the myocardium showed areas of calcification necrosis quite identical with those Dr. McJunkin has produced experimentally. There is a striking similarity between the effects on the heart of phosphatemia produced by these developmental defects and the changes he produces in experimental animals.

(Dr. William Boyd, Winnipeg.) I should like to ask Dr. Mallory if he has anything to say about the possibility of a reversal of the action, with the parathyroid activity being the primary cause of the renal lesion, rather than the changes in the parathyroid being secondary. I have in mind some experiments which Dr. Bruce Chown of Winnipeg performed recently. He found that injections of parathyroid extract caused subepithelial deposits in the renal tubules, and that these deposits pushed the lining epithelium forward to such a degree that tubular blockage occurred, so that many of the animals died of renal insufficiency. It would appear that parathyroid overactivity can cause renal insufficiency, in addition to renal insufficiency causing parathyroid hyperplasia.

(Dr. Sheldon A. Jacobson, New York City.) Those who have worked with the thyroid and its disorders have in many cases long had the feeling, as is well known, that the thyroid dysfunction might not be primary in Graves' disease, but might possibly be secondary, and the various anatomical changes with function of the thyroid in diseases other than Graves' disease lend some support to this view. There is no doubt that hyperparathyroidism itself can produce a picture similar to that of von Recklinghausen's disease. Whether it is identical is a question, and there have been a few workers who have been most interested in that field who have been of the opinion that a somewhat analogous relationship exists—that the parathyroids may be involved secondarily to some unknown factor. Such observations as those reported lend some additional weight to that view.

(Dr. Mallory.) In answer to Dr. McJunkin, in all these cases we had routine sections of the myocardium and I did not notice any acute myocarditis. We did not cut many blocks, or look particularly hard for it, however.

Dr. Wells spoke of renal rickets, and that, to our mind, is exactly the same syndrome as that I have shown here, the only difference being that it occurs

in a child before the epiphyses have united rather than in an adult. It is a question whether such cases really should be regarded as rickets at all.

As Dr. Boyd pointed out, there is a theoretical possibility of a completely reversible interrelationship: overactive parathyroids can produce renal insufficiency as the result of calcium deposits in the kidneys, and a primary renal insufficiency can produce hyperplasia of the parathyroids and probably real hyperparathyroidism. In 1 case of renal rickets Schelling showed there was an increased amount of parathyroid hormone in the blood, and the bone changes in these cases are indistinguishable from those of primary hyperparathyroidism. It is possible to start at either end of the picture and wind up with the end-stage, and for that reason the possibility of distinguishing simply from the morphology of the parathyroids the two types of hyperplasia has practical significance. In some cases with an inadequate history we might have difficulty in doing it on clinical grounds.

In reply to Dr. Jacobson's question whether this disease is primary in the parathyroid or not, I can only say that certainly some of the cases are not primary. This postnephritic group is certainly not. I do not think that the diffuse hyperplasias of the water-clear type are primary either; the fact that after doing a subtotal parathyroidectomy the disease continues and the defect occurs again would point to its not being primary. We feel sure it is outside the parathyroid but have no idea of the etiology. In the group where the changes in the parathyroid glands are localized our experience at the Massachusetts General Hospital has been in every instance that removal of the localized nodule has resulted in an apparently complete cure. Most of our cases have not run longer than 2 or 3 years, but we have several cases of 5 years duration without recurrence. Therefore this group of cases we feel represents primary disease of the parathyroid organ itself.

#### MYOCARDIAL HYPERPLASIA IN CARDIAC HYPERTROPHY OF INFANCY. H. Edward MacMahon, Boston, Mass.

*Abstract.* The statement that "in myocardial hypertrophy the increase in size of the heart is due solely to an increase in size of the individual muscle fibers, and is not the result of a multiplication of these fibers" has been so frequently repeated in textbooks and in journals that it may now be considered as practically axiomatic. This is based primarily on two indisputable facts in respect to the fully mature heart: first, careful measurements of the muscle fibers of hypertrophied hearts have shown that the increase in size of hearts may be explained mathematically on the basis of an increase in size of the individual myocardial fibers; and secondly, patient search through the myocardium in cases of cardiac hypertrophy has failed to reveal any positive evidence, in the form of mitoses, of true myocardial proliferation—a fact that is strongly supported by the lack of regenerative power of heart muscle fibers following severe injury.

The purpose of this paper is not to contradict but rather to modify the above mentioned statement by pointing out that in the infant, at least until the end of the 2nd year, cardiac hypertrophy, whether primary or secondary, may be accompanied, not only by an increase in size of the individual fibers, but by an active proliferation of these as well.

In a careful examination of the myocardium of infants whose hearts were

definitely increased in weight, comprising cases of so-called primary idiopathic hypertrophy, as well as those of left ventricular hypertrophy of renal and aortic origin, it has been possible to demonstrate mitotic figures in varying stages of nuclear division. No part of the ventricular myocardium has been particularly favored, though they appear to occur more often in the outer portions of the myocardium and toward the tip of the left ventricle. It is possible that this should not be considered as an example of true hyperplasia but merely an acceleration of a normal physiological process in which the child's heart at this age is still undergoing a natural histiogenesis.

It is difficult to determine at what age the child's heart may be considered to have acquired its full complement of myocardial fibers, but the paucity of mitoses in average sized hearts of children during the first 2 and 3 months of life is so commonly recognized as to suggest that the active proliferation of muscle elements under ordinary conditions ceases quite early.

Since growth of the myocardium by the active proliferation of muscle elements may be demonstrated in the enlarged hearts of infants in the 1st and 2nd years, it is possible that during this same period myocardial regeneration may occur as well.

### *Discussion*

(Dr. Robert A. Moore, New York City.) I should like to ask Dr. MacMahon if he has any quantitative observations similar to those made by Drs. Karsner, Saphir and Todd on the hypertrophic adult heart. We have recently studied a case of idiopathic hypertrophy of the heart in a 1 year old infant, not of the von Gierke type, in which, using these methods and counting the number of fibers in a large number of fields we could find no evidence that there was an increase in the number of muscle fibers and no increase in the number of nuclei.

(Dr. MacMahon.) In answer to Dr. Moore's question in regard to the hypertrophy of the muscle fibers in infants' hearts, I can say that one does find a true hypertrophy of the muscle fibers in hypertrophied hearts of infants. One finds this whether the hypertrophy is of the primary idiopathic type or whether it is associated with deposition of glycogen or secondary to hypertension, coarctation or renal disease. It is awfully difficult to attempt to estimate mathematically the weight of a child's heart by measuring the width of the muscle fibers alone, for these vary so in size and at times one finds hypertrophied fibers and less developed fibers lying side by side. I feel that in addition to hypertrophy that is present in these cases, the possibility of a true proliferation of heart muscle fibers should not be entirely overlooked, especially at this very early age.

THE HISTOPATHOLOGY OF EXPERIMENTALLY PRODUCED ENDOCARDITIS. Alexander Nedzel (by invitation), Chicago, Ill.

*Abstract.* Pitressin injections cause a rise in blood pressure by stimulating the blood vessels by peripheral action, evoking the constriction of the finer arterioles and capillaries. Periods of high blood pressure (ARS phase of Petersen) are followed by periods of low blood pressure, because with a period of spasm an anoxemia prevails in certain tissue; this causes stimulation, products of incomplete tissue metabolism are released and thrown into the

circulation, capillaries are dilated ( $\text{CO}_2$  accumulates) and the diastolic blood pressure falls.

The increase in pressure will mean also more work for the left heart, because the margins of the valves will impinge upon each other more forcibly and as a consequence the marginal endothelium in such a region will be mechanically stimulated to a greater degree.

There are also coincident changes in the vessels of the valves. The pitressin injections which produce pressure response are very frequently followed by hemorrhage into the leaflets of the valves, causing considerable nutritive changes in the tissue of the valves.

After the pitressin injections we note the gradual changes in the valvular endothelium. The endothelial nuclei, at first, appear somewhat shorter and thicker. Their relation to the subendothelial layer changes with the thickening of the endothelial surface, on account of a thickening of the cytoplasm. Further, the cytoplasm of the endothelium definitely swells more and the nuclei no longer project above the surface. The nuclei become oval and round and later they appear elongated again, but perpendicular to valvular surface.

The smooth valvular surface also changes, becoming roughened and interrupted. Some of the endothelial cells appear to show vacuolization and their attachment to the subendothelial layer to be less firm. These vacuolated cells begin to project freely on the surface, being loosely attached to the subendothelial layer. The subendothelial cells are quite edematous.

Occasionally we actually find bacterial inclusions in heart valves in dogs that have had the induced pressor episodes.

The described changes in valvular endothelium cause the latter to become adhesive and bacteria circulating in the blood find an opportunity to adhere to the valvular surface and invade the valve. So if we, after pitressin, inject bacteria, we may see them adhering to the valvular surface and find them by cultural and bacteriological methods.

Cultural and bacterioscopical findings are accompanied by macroscopic lesions. At first a slightly elevated, hyperemic round lesion of a size from pinpoint to that of the head of a pin is found on the valvular surface. This type of lesion may be multiple and confluent. Later button-like excrescences and widely spread hemorrhagic zones appear. Finally small vegetations and ulcers develop.

If no bacteria were introduced after the pitressin injection, in a vast majority of cases the dogs appeared healthy, showing no signs of illness (except the reactions immediately after pitressin injections). But in some of the killed animals macroscopic and microscopic changes were observed.

At first a hemorrhagic lesion is found in the subendothelial area. The surrounding tissue appears swollen and edematous, with dilatation of vascular channels. An appearance of round cells and initial stage of fibroblastic proliferation are noted. Later, beneath the valvular surface there are observed a congregation of small and large mononuclears and fibroblasts. Some of the cells show vesicular nuclei. These cell clusters resemble an Aschoff nodule. We also observe typical palisades of fibroblasts, perpendicular to the valvular surface. Some of these fibroblasts proliferate above the valvular surface in a manner resembling the initial shape of verruca.

If the dogs are sacrificed in several weeks or months we find then congregations of scar tissue on the valvular surface in the form of a small verruca-

like formation. In some places the fibroblasts in palisade formation and perpendicular to the valvular surface bulged out of the valvular stroma, forming leaf-like bodies on the surface.

We also find bundles of scar tissue extending from the deeper tissue of the valve to the surface. Some of them are parallel to the valvular surface. There are also areas with no formed elements and only occasional round cells are observed. There are also found small verrucae whose lower portions are composed of fibrous tissue with few, if any, formed elements. At the base we might find bundles of scar tissue.

The fundamental and initial changes in the heart valves of animals with bacterial or non-bacterial endocarditis are the same in both cases. After mere pressor episodes, not accompanied by simultaneous bacteremia, the heart valve shows pathological changes resembling changes in rheumatic endocarditis. The bland endocarditis is a local reaction of a macroorganism to changes in its vascular status. The bacterial endocarditis represents a functional stage where bacterial adhesion and proliferation have been added to the primary change in endothelial status.

### *Discussion*

(Dr. Alan R. Moritz, Cleveland.) I should like to ask Dr. Nedzel how he knew that the hemorrhage in the substance of the valve was from a vessel in the valve. We see hemorrhages in avascular portions of valves in man occasionally, and I would be interested to know how frequently the valves in dogs' hearts are vascularized.

(Dr. Benjamin J. Clawson, Minneapolis.) I should like to ask about the blood vessels deep in the valves, before you gave the inoculation with bacteria, and I should also like to ask about the interpretation of the pathogenesis of the disease, whether it develops outside the valve, or whether the infection begins deep in the valve. I was not certain from the sections whether the inflammation extended deep in the valve, as is commonly found.

(Dr. Isabella H. Perry, San Francisco.) Was the vitamin C intake in these animals lower in winter?

(Dr. Burton R. Rogers, Chicago.) I should like to ask Dr. Nedzel how he explains the heart beating 70 times a minute, and the flood of blood constantly washing over these valves, and the tenacity of these bacteria to stick on the free surface of the valve. Is there a possibility that they really come around when the aortic contracts and drives the blood into the coronary arteries and into the capillaries between the two transparent serous layers of the valve? Don't they really sneak in by the back door to the subserous side? I have often noticed over-exerted cattle driven rapidly, the whip and horses acting similarly to pitressin and increasing heart action, show endocardial congestion of valves and ventricles.

(Dr. Nedzel.) In many slides where hemorrhages were observed we could actually find many dilated blood vessels and we could see these hemorrhages coming out of blood vessels.

I did not look into the vitamin C deficiency. Our animals were fed well all year round. The animal hospital has an especially trained personnel and the animals have a well balanced diet. I do not think that the vitamin deficiency has been an important factor in our experiments.

In reply to Dr. Clawson's question about the blood vessels, I could not see them in the animals before injection of pitressin, because the observations were carried out after sacrificing the dogs. In control animals there were hardly any blood vessels seen. They were observed at the base of the valves, but after pitressin injections we could see dilated blood vessels also in other parts of the valves. I am sorry that time did not permit me to show these dilated blood vessels, but I may state that the vascular response even without the injection of bacteria was very noticeable. I am convinced that in my experiments the bacterial localization occurred from outside from the blood stream; bacteria, first settling on the valvular endothelium, gradually penetrated into deeper layers.

(Dr. Clawson.) About the inflammation of the valves?

(Dr. Nedzel.) In general we can say the heart valve *in toto* is edematous. I was primarily interested in the endothelial changes in the initial stages in connection with localization of the bacteria on the valvular surface. The said endothelial changes were of such nature that here was prepared a surface to which the bacteria could adhere and multiply. I think that also answers Dr. Rogers' question. In my experiments bacteria settled on the valves from the blood stream. They were constantly found on the valvular surface and did not come as emboli through the coronary blood vessels.

#### EXPERIMENTAL PULMONARY EDEMA AND CONGESTION. Sidney Farber, Boston, Mass.

**Abstract.** This report serves as an introduction to a study of neuropathic pulmonary edema in laboratory animals and in man. Section of both cervical vagosympathetic nerves initiates a series of changes which lead to death (Schiff, Traube, Schafer) usually within  $2\frac{1}{2}$  to 4 hours in the guinea pig (250 to 700 gm.) and 8 to 24 hours in the rabbit (800 to 3500 gm.). Severe pulmonary edema, congestion and sometimes hemorrhage are the most constant important findings. Consequences of the coincidental laryngeal paralysis (slow asphyxia, particularly in young animals; aspiration of secretions and food) may be superimposed on the pulmonary edema in variable degrees, giving a picture similar to that found in certain instances of poliomyeloencephalitis. Unilateral vagotomy causes neither pulmonary edema nor death. The survival time is prolonged (3 to 4 times) in the guinea pig if an interval of more than 10 days exists between section of the two nerves.

When the complications secondary to laryngeal paralysis are prevented (cannulation of the trachea or continuous artificial respiration) severe pulmonary edema still develops; the survival time is somewhat prolonged in the rabbit, but remains unaltered in the guinea pig. Laryngeal paralysis, therefore, is not an essential factor in the causation of pulmonary edema and death following double cervical vagotomy. That the pulmonary branches of the vagosympathetic nerves are of primary importance is demonstrated by the occurrence of edema and death when only those branches are destroyed (guinea pig).

Preliminary studies of acute pulmonary edema in man indicate that disturbances to the vasomotor control of the pulmonary vessels, caused by either central or peripheral nerve disease, may account for alterations in the dynamics of the pulmonary circulation and in the integrity of the vessel walls. These

alterations are of importance in the pathogenesis of one form of pulmonary edema in man.

### *Discussion*

(Dr. Virgil H. Moon, Philadelphia.) I am extremely interested in Dr. Farber's presentation on pulmonary edema for the reason that a year ago it was my privilege to present before this Association the results of experiments on the production of edema. In these experiments agents were used that had the capacity of altering the permeability of the capillary walls. These agents included extracts of normal tissues, bile, sodium glycocholate, and narcotic substances, such as barbiturates. In each instance in which shock was produced and death was delayed, pulmonary edema was present. We came to the conclusion that the etiology of one type of pulmonary edema is integral with the etiology of shock. It has been our problem to determine the origin of shock when there were lesions of the central nervous system which had been observed. The shock syndrome may develop following a brain tumor, hemorrhage, or some other type of injury to the brain. Postmortem examination in such cases shows the same type of changes that we find characteristic of shock otherwise produced. This leads to the conclusion that one of the mechanisms whereby the vascular system may lose its tonus and congestion and edema of the lungs may develop, is of central nervous origin, or at least is bound up with the functioning of the central nervous system. I am therefore interested in the observation of Dr. Farber that permeability of the capillary walls was a mechanism in the production of pulmonary edema in his experiments. I am quite sure he is correct in that. I should like to ask whether barbiturate anesthesia was used in his animals. Our experiences have led us to abandon that type of anesthesia entirely in any type of experiment in which capillary tonus is a factor.

(Dr. H. Edward MacMahon, Boston.) Dr. Farber has told us that by cutting the vagus nerves bilaterally, pulmonary edema results. I should like to know if a unilateral pulmonary edema may be produced by cutting the vagus on one side only. Clinically and at the autopsy table one meets unilateral congestion, edema and hemorrhage at times following cerebral injury or associated with more severe operations. The explanation of these has been on a neurological basis and such terms as "neuropathic congestion," "neuropathic edema," and "neuropathic bleeding" are used. It would be of great interest if these conditions could be reproduced by mere unilateral section of the vagus nerve.

(Dr. Burton R. Rogers, Chicago.) I should like to ask Dr. Farber if he might also have cut the sympathetic nerves that go down the neck in the same sheath, on both sides along with the pneumogastric or vagus. They are considered antagonistic to the vagus.

(Dr. Farber.) I am well acquainted with Dr. Moon's interesting work on pulmonary edema. It should be emphasized that the experiments reported today deal with only one of the several causes of acute pulmonary edema. We have used barbiturate anesthesia in some experiments and we can verify what Dr. Moon has observed. Acute pulmonary edema was produced, however, under our experimental conditions when a variety of anesthetic agents were used, including local skin anesthesia. From experiments already carried out, but not reported today, it appears certain, in response to Dr. Moon's question,



that increased permeability of the pulmonary capillaries is of great importance in the production of this form of edema.

Dr. MacMahon asked about unilateral vagotomy. Unilateral vagotomy, either right or left, is followed by neither pulmonary edema nor death. The animals survive for many months without demonstrable changes in either the lungs or in general health. It is true, however, as we shall show on another occasion, that certain changes do occur following unilateral vagotomy which lead to pulmonary edema when other factors are introduced.

We have investigated the changes in the lung following thoracic sympathectomy and are continuing our work at the present time. I am not certain that it is correct to speak of a strong antagonism between the sympathetic and the parasympathetic nerves as far as vasomotor control of the pulmonary vessels is concerned.

#### MECHANISM OF INCREASED CAPILLARY PERMEABILITY IN INFLAMMATION. Valy Menkin, Boston, Mass.

**Abstract.** Previous studies of the writer have indicated the presence of a factor in various types of exudates which is capable of promptly increasing the permeability of the capillary wall (*J. Exper. Med.*, 1936, 64, 485). The liberation of this substance by injured tissue, as evidenced by its consequent recovery in the exudate, has offered a reasonable explanation for the mechanism of increased plasma filtration into an inflamed area. The factor exhibited none of the manifest properties of histamine or of its presumably closely related H-substance, thereby rendering it difficult to accept the views of Lewis on the subject. The active substance was shown to be dialyzable and was recovered in a crystalline protein-free form.

An attempt was made to isolate the active factor relatively free of any gross impurities. Exudates were for the most part obtained from dogs after intrapleural injection with turpentine. However, in 1 case an exudate resulting from the intraperitoneal injection of aleuronat and starch in a rabbit was also analyzed for the presence of the active permeability factor. The cell-free exudate was treated with pyridine and the active principle was extracted after prolonged stirring of the precipitated mixture. This was eventually centrifugalized and the supernatant fluid was treated with several times its volume of acetone. The precipitate was centrifuged off and the supernatant fraction was evaporated to dryness *in vacuo*. Treatment of this residue with distilled water, followed by filtration and evaporation, yielded a practically homogeneous crystalline material. These crystals were characterized by a peculiar "branched and notched" pattern which sometimes assumed a "cross-like" appearance.

The crystalline material is extremely active (1:1000) in inducing a prompt increase in capillary permeability in rabbits when it is introduced intracutaneously, as evidenced by the rapid local accumulation of dye from the circulating blood stream. The crystals are very hygroscopic; they are soluble in water, acetone and aqueous alcohol, and relatively insoluble in ether and absolute alcohol. The material is heat stable. It yields a negative biuret test for proteins. The Molisch and Fehling tests for carbohydrates are negative. Unlike histamine (M. P. 130°C.) it shows no sharp melting point. At about 175°C. it chars and at 265°C. to 298°C. it still has not melted. The available evidence from proteolytic studies of total protein nitrogen and amino acid

nitrogen indicates that it is an intermediary product of protein catabolism. Its nitrogenous content averages 2.3 per cent. The pH of its aqueous solution may be slightly acid (6.5) or slightly alkaline to phenol red. It is precipitated out by saturated  $(\text{NH}_4)_2\text{SO}_4$ . It is readily dialyzable. It appears unlikely on the basis of accumulated observations that it is either a proteose, a peptone, or an amino acid. The data available at present seem to indicate that this active substance probably belongs to the group of polypeptides. Further work is being conducted in an endeavor to ascertain complete purity of the isolated substance and to determine its precise chemical structure.

This active substance recovered from exudates has in addition the interesting property of inducing a prompt aggregation and migration of polymorphonuclear leukocytes through the endothelial wall of capillaries. Within 20 to 40 minutes after its intracutaneous inoculation microscopic sections of the tissue reveal small vessels literally crowded with polymorphonuclear cells actively migrating outward. Furthermore, the substance is positively chemotactic. When placed in capillary glass tubes sealed at one end and introduced into an inflamed peritoneal cavity, the tubes soon become filled with leukocytes. This prompt effect on the migration of leukocytes following intracutaneous injection does not seem to depend primarily on its initial effect in increasing capillary permeability, for the introduction of turpentine, although causing an intense and prompt increase in endothelial permeation, fails to induce the rapid leukocytic migration observed in the case of the active substance. When normal blood is treated with the same extraction procedure a crystalline material is likewise recovered, but apparently in reduced concentration. It is only slightly active in inducing increased capillary permeability, and it barely calls forth any migration of leukocytes in equivalent intervals. The untreated exudate also induces a local cutaneous aggregation of leukocytes.

For the sake of convenience the name *leukotaxine* is tentatively proposed for this active crystalline nitrogenous substance which is evidently released by injured tissue and is readily recovered in inflammatory exudates. "Leukotaxine" *per se* is capable of rapidly initiating the usual sequences of the inflammatory reaction by first inducing a prompt increase in capillary permeability and secondly by causing an extremely rapid aggregation and migration of leukocytes through the endothelial wall.

### Discussion

(Dr. Robert J. Parsons, New York City.) I should like to ask what the action of this material is when injected into the tissues with dyes or with diphtheria toxin. I am thinking of the Duran-Reynals factor.

(Dr. Menkin.) We have of course been very much interested to know whether the permeability factor in inflammation is related in any way to the Duran-Reynals factor, but we find that it is evidently an entirely different substance. The Duran-Reynals factor is non-dialyzable; it is heat-labile, whereas the permeability factor is dialyzable and heat stable. We have studied testicular extract and found that we could dissociate, by thermostability criterion, the two factors. We found, interestingly enough, that in normal testicular extract both factors are present—the Duran-Reynals factor and the permeability factor found in injury (*i.e.*, leukotaxine).

VASCULARITY OF THE BLOOD VESSEL WALL: PATHOLOGICAL CHANGES. Milton C. Winternitz and (by invitation) Robert M. Thomas and Philip M. LeCompte, New Haven, Conn.

*Abstract.* Intrinsic vessels arising from three separate sources are demonstrable in the aorta of the cow; they come from the adventitia, from the lip of the orifice of each branch, and also arise directly from the intima. These three groups anastomose freely within the wall of the vessel. Similar patterns are demonstrable in man and other animals. In normal young human vessels they are seen only occasionally, but with increasing age they become much more evident. They become conspicuous in physiological obliteration of vessels such as the ductus arteriosus and in the slow occlusion of the vessels in or adjacent to tuberculous cavities.

In the occlusion of the ductus arteriosus blood may be trapped in the lumen, or in the intrinsic vessels of the wall. This is the source of the concretions that are encountered as early as 4 months after birth. In the proliferation of the intima associated with the forms of disease that are included under the term arteriosclerosis, vascularity plays a conspicuous role. The number and size of blood vessels in the connective tissue matrix vary greatly. Blood may become trapped in these vessels, as occurs in the wall of the obliterated ductus arteriosus, to form concretions. Hemorrhages of varying size also occur and these lead in turn to the changes commonly classified as atheroma.

ENVIRONMENTAL FACTORS IN THE THROMBOTIC CONSTITUTION. William F. Petersen, Chicago, Ill.

*Abstract.* Apart from the factors in thrombosis that are generally accepted, such as changes in the vessel walls, in the blood constituents, and in the rate of flow, certain intangible and apparently unpredictable factors appear to take part in the constellation underlying the vascular condition. That seasonal forces must be considered has become evident from the statistics of the distribution of cases of thrombosis during the year; geographic considerations have revealed that thrombosis is rare in the tropics by comparison with the northern latitudes.

In a study of definitely dated thrombosis (retinal) in patients under continuous detailed chemical and clinical study over long periods of time, it has been made probable that an immediate environmental influence of the air mass plays a distinct role, particularly in providing vasomotor changes that are apparently summative in effect.

The precipitation of the thrombosis occurs definitely with the COD phase (*i.e.* the metabolic status that follows periods of unusually high blood pressure) when tissue anoxia has led to great periods of production of anoxybiotic acids, to capillary stimulation, and to dilatation of the vascular bed.

Such phase amplification occurs occasionally during the course of the repeated passage of abrupt polar fronts and when the reactive phase is augmented by an unusual rise in environmental temperature, the opportunity for thrombosis is definitely enhanced.

EXPERIMENTAL STUDIES ON THE INFLUENCE OF CARBON DIOXIDE ON THROMBOSIS AND OF CARBON DIOXIDE AND OXYGEN ON BLOOD CLOTTING. Joseph Tannenbergh, Albany, N. Y.

*Abstract.* The studies concerning the influence of carbon dioxide on formation and progression of thrombosis were made on the exposed and narrowed inferior vena cava of rabbits. Beginning at various days following the operation (from the 1st to the 12th day) the rabbits were exposed to several periods of breathing mixtures of carbon dioxide. From one to five periods having the maximum duration of 8 hours were applied. The rabbits were killed at from the 2nd to the 18th day following the operation. Thrombi were found at the narrowed sites of the veins. The histological structure, however, showed that they were formed at one period referable exclusively to the time of operation. No additional proliferation corresponding to the breathing periods of carbon dioxide was present.

A new apparatus for the study of blood clotting under the influence of various gases was constructed which permitted keeping blood at constant temperature and under varying pressure of different gases without undue mechanical irritation during the clotting process. By means of this apparatus the influence of carbon dioxide and oxygen was studied on clotting of blood obtained from various sources. Gas pressure of from 10 to 120 mm. of mercury was applied. Carbon dioxide produced a marked delay of the clotting rate of from 30 to 50 per cent, but was not able to prevent the clotting entirely. Oxygen, even under pressure of 120 mm. of mercury, produced only a slight acceleration of clotting compared with atmospheric air. In additional experiments the influence of carbon dioxide on the agglutination of blood platelets was studied and found corresponding to the delay of blood clotting. The carbon dioxide is considered one, but not the only or most important, metabolic product that counteracts blood clotting and thrombosis, especially in the small veins of parenchymatous organs, such as the liver and kidneys, where the blood having passed two capillary systems is flowing particularly slowly.

ARTERIOLEAR DISEASE IN HYPERTENSIVE AND NON-HYPERTENSIVE PATIENTS. Alan R. Moritz and (by invitation) M. R. Oldt, Cleveland, Ohio.

*Abstract.* The histological types, relative severity and distribution of chronic arteriolar lesions were investigated in all available tissues and organs from 200 autopsies. These 200 cases were comprised of two groups of 100 each, one group being of known non-hypertensive individuals and the other group of persons known to have had chronic hypertension. The tissues were examined objectively and the kind and severity of the changes in arterioles (100  $\mu$  or less in external diameter) were recorded.

The only portion of the body in which the presence of moderate or severe arteriolar sclerosis was almost invariably associated with evidence of chronic hypertension, whereas the absence of arteriolar sclerosis was almost invariably associated with evidence that chronic hypertension had not been present, was the kidney. Chronic arteriolar lesions were found in many situations in non-hypertensives and the severity of the lesions tended to increase with age. When, however, the arterioles of the kidney were affected in the same manner chronic hypertension was almost invariably associated. These findings indicated

either that chronic hypertension was caused by renal arteriolar disease, or that the renal arterioles had an unusually specific susceptibility to the effects of hypertension. If the examination were restricted to the non-hypertensive group it appeared that the renal arterioles were not especially predisposed to disease as compared with those of the spleen, pancreas and adrenals. If the occurrence of renal arteriolar sclerosis in hypertension represented effect of the hypertension, it would be reasonable to expect the renal arterioles to be susceptible to other injuries and to have a relatively high incidence of occurrence in non-hypertensive as well as hypertensive individuals, which was not the case.

The examination of tissues and organs where the presence of arteriolar disease was not necessarily accompanied by hypertension showed that in cases of hypertension both the incidence and severity of the arteriolar lesions were augmented. This was in accord with the clinical and experimental evidence that chronic hypertension may cause or augment arteriolar sclerosis.

This investigation did not disclose evidence that chronic hypertension was invariably associated with renal arteriolar disease, or that renal arteriolar disease invariably caused chronic hypertension. It did lend support to the view that in many cases of chronic hypertension the primary change was renal arteriolar sclerosis and was in accord with Goldblatt's experimental observations that reduction in blood flow through the kidney causes hypertension.

Three histological types of chronic arteriolar disease were identified and described. These were (1) intimal hyalinization; (2) medial hyperplasia; and (3) endothelial hyperplasia. These occurred in various combinations and were frequently altered by secondary degenerative changes. No type of chronic arteriolar lesion was found to be of and by itself pathognomonic of hypertension.

### *Discussion*

(Dr. H. Gideon Wells, Chicago.) Dr. Moritz did not call attention to the striking similarity in his graphs of the arteries of the adrenal and kidney. That I think is pretty well recognized by pathologists in general. In routine examinations of autopsies, when I get the adrenal before the kidney, and have looked over the arteries of the adrenal, I can tell what I will find in the kidney. Dr. Moritz says that these changes in the kidney cannot be disregarded as indicating a relation of the kidney to hypertension, and you cannot very well leave out the adrenal either.

(Dr. E. T. Bell, Minneapolis.) Dr. Moritz has given an interesting explanation of this disease, and some splendid pictures of the structural changes in the vessels. I think that some of the differences of opinion among us are due to the fact that we draw the line differently on what an arteriole is. When one includes the small arteries with the arterioles he will find more arteriolar sclerosis than if he uses only the afferent glomerular arteriole. In all my studies I have used the afferent glomerular arterioles only, and that is perhaps the reason why I have found some cases of known clinical hypertension that did not have arteriolar sclerosis. My reports show that about 10 per cent of cases of clinical hypertension show no sclerosis of the afferent glomerular arterioles. If the arteriolar disease is first, and the hypertension secondary, how are we going to explain the 10 per cent with no arteriolar disease? On

the other hand, if you maintain that hypertension comes first and causes the arteriolar disease, you are still faced with the difficulty of explaining the cases where arteriolar disease is not present. Neither hypothesis escapes the dilemma. Dr. Moritz advances the explanation offered by Theodore Fahr that the primary disease is in the arterioles and that hypertension is secondary to arteriolar disease. Fahr asks: if the hypertension is primary, why does it affect only the kidney arterioles? But Fahr also assumes a hypersusceptibility of the renal arterioles to the agent that causes arteriolar sclerosis.

(Dr. Moritz.) I think that Dr. Wells' question might be answered in the same way if he had chosen the spleen instead of the adrenal. Severe arteriolar sclerosis occurs in the spleen and adrenals of hypertensive individuals, but it also occurs frequently in both organs in non-hypertensives. On the other hand, renal arteriolar sclerosis is rarely seen in non-hypertensives and in this investigation it was found almost invariably in cases of chronic hypertension.

In answer to Dr. Bell, I do not feel that disease in an arteriole of 100  $\mu$  in diameter is any less significant than disease in an afferent arteriole of 18  $\mu$  in diameter.

**THE PATHOGENESIS OF ICTERUS NEONATORUM.** Graham Ross (by invitation), Theodore R. Waugh and (by invitation) H. Tait Malloy, Montreal, Canada.

*Abstract.* A comparative study of the amount of blood destruction and bile pigment excretion during the 1st week of life of jaundiced and non-jaundiced infants was carried out. These investigations showed that:

1. The amount of excretion of bilirubin and urobilin in the stools during the 1st week was greater in the non-jaundiced cases.
2. The average level of the hemoglobin and total corpuscle volume of the blood on the 4th or 5th day was essentially the same in the two groups, though there were marked differences in individual cases.
3. The van den Bergh reaction on the blood plasma was always of the direct delayed type and quantitatively was proportional to the degree of jaundice.
4. Bilirubin was present in the urine in the cases showing jaundice and tended to appear when the bilirubinemia rose over four units.
5. The average fall in hemoglobin and total corpuscle volume from the 1st to the 5th day was essentially the same in both groups.

These findings offer a strong refutation to the hemolytic hypothesis of icterus neonatorum. Jaundice of the newborn is undoubtedly made possible by the blood destruction which occurs at birth. Nevertheless, whether a child does or does not develop jaundice is due to some factor other than the amount of destruction which occurs at this time.

### *Discussion*

(Dr. E. B. Krumbhaar, Philadelphia.) If one thinks of the state of the blood at a given moment as indicating a state of hemolytopoietic equilibrium, would it not be possible that Dr. Waugh's figures would be compatible with another situation, namely that although there is an excessive destruction of blood the levels are still equal, as were shown, because the destruction is

excessively well compensated for by increased bone marrow output? If this compensation were thought of as accomplished with little or no strain on the bone marrow, practically no change in the peripheral blood picture would have to be expected.

(Dr. Waugh.) In answer to Dr. Krumbhaar, there is no evidence in the blood in jaundiced or non-jaundiced babies, so far as I know, of any excessive hematopoietic activity in 1 case as compared with the other. As a rule, the nucleated red cells disappear quite rapidly, along about the 3rd or 4th days, and so far as I know, it has never been pointed out, although it may be possible, that there does exist any hematological difference.

AN EXPERIMENTAL STUDY OF THE EFFECT OF BENZEDRINE SULPHATE ON RATS. William E. Ehrich (by invitation) and E. B. Krumbhaar, Philadelphia, Pa.

*Abstract.* One hundred and seventy-one albino rats, weighing from 50 to 385 gm. were used. One hundred and forty-five were injected subcutaneously with doses of 1 to 500 mg. per kilo from 1½ to 6 weeks. The lethal dose decreased with the weight (age) of the rats. In animals of 50 to 95 gm. it amounted to 200 mg.; in rats of 100 to 195 gm., to 50 to 60 mg.; in rats of 210 to 385 gm., to 30 to 35 gm. per kilo. After repeated injections a slightly increased tolerance was noted. If 5 mg. and more were given the rats showed excitement, diarrhea, mydriasis, transient inhibition of weight increase, erythrocytosis, leukocytosis, and so on. With 25 mg. and more they showed an increase in glycogen and a decrease in fat in liver and fat tissue. In rats that died from the drug frequent changes were constrictions of the small intestine, congestion of the liver, either marked constriction or congestion of the spleen, and subpleural hemorrhages in the lungs. Rats that died of from 30 to 100 mg. of benzedrine showed necroses in liver, spleen and kidneys, whereas those that died from higher doses did not. Lesions in the myocardium, arterial or intestinal walls, such as are observed in chronic adrenalinism, were not found.

INHIBITION OF THE SHWARTZMAN PHENOMENON IN LYMPH NODES. Lewis Henry Koplik (by invitation), New York City.

*Abstract.* Gross (*Zentralbl. f. Bakt.*, 1. Abt., Orig., 1931, 122, 96) and Ogata (*J. Exper. Med.*, 1936, 63, 59) observed that the intravenous injection of a potent bacterial filtrate given within a certain period of time prior to or following the skin preparatory injection produced an inhibition of the Schwartzman phenomenon. The association of hemorrhage and thrombosis in regional lymph nodes with the Schwartzman phenomenon has already been described (Koplik, L. H., *J. Exper. Med.*, 1937, 65, 287). It was pointed out that this reaction, while limited to the regional nodes, may be independent of hemorrhage at the injected skin site, the lymph nodes being more susceptible to the production of the Schwartzman phenomenon than the areas of skin which they drain. The purpose of the present work was to study the reaction in regional and distant lymph nodes of rabbits in which the inhibition of the Schwartzman phenomenon had been attempted according to the method outlined by Ogata.

Stock rabbits were given an intravenous injection (1 cc. per kilo) of dilu-

tions of meningococcus (Shwartzman, G., *J. Infect. Dis.*, 1929, 45, 232), *B. coli*, or *B. typhosus* culture filtrate (Shwartzman, G., *Proc. Soc. Exper. Biol. & Med.*, 1929, 26, 843) immediately preceding the skin preparatory injection for the Shwartzman phenomenon. The dilution of meningococcus culture filtrate was equivalent to 20 to 25 reacting units per cc., that of the *B. coli* and *B. typhosus* culture filtrates varied between 1:10 and 1:75 depending on the strain and filtrate used. Meningococcus culture filtrate was used for the production of the Shwartzman phenomenon, 0.25 cc. of a 1:25 dilution for the preparatory injection and 20 to 25 reacting units for the provocative intravenous injection given 20 to 24 hours later. Four to 5 hours after this second intravenous injection the skin reactions were read and the animals were killed by ether. They were autopsied, and regional and distant lymph nodes were removed for study. Control rabbits were similarly treated for the production of the Shwartzman phenomenon but did not receive the preliminary inhibitory injection of filtrate.

Of 16 control rabbits 15 showed gross and microscopic hemorrhage and all presented extensive thrombotic changes in the veins and capillaries of the regional lymph nodes. The injected skin sites were negative in 5 instances; in 11, the Shwartzman phenomenon was present. Distant lymph nodes were normal.

In contrast, rabbits given an intravenous injection of 20 or 25 reacting units of meningococcus culture filtrate just before the skin preparatory dose showed neither hemorrhage nor thrombosis in the regional nodes in 7 of 9 animals so treated. The skin reactions were inhibited in 5 of these rabbits. Distant nodes were negative in all cases.

Inhibition of the Shwartzman phenomenon in regional lymph nodes was also observed in rabbits given an intravenous injection of heterologous filtrate (*B. coli* or *B. typhosus*) just before the skin preparatory injection. Certain concentrations of filtrate in the inhibitory injection, depending on the strain and filtrate used, were necessary for inhibition of the phenomenon. Thus, with a dilution of 1:30 of a filtrate of *B. typhosus* (strain TL) culture lymph nodes were negative in all of 4 rabbits, while a dilution of 1:75 evoked no inhibition in 6 others. In these experiments the skin reactions were also inhibited though not so regularly or completely as those in the lymph nodes.

The experiments demonstrate that it is possible to prevent the appearance of thromboses and hemorrhage in regional lymph nodes by an inhibitory intravenous injection of a bacterial filtrate just prior to the preparatory intradermal injection. Just as there is a lower threshold for the production of the Shwartzman phenomenon in lymph nodes than in the skin, so the results of the present experiments are suggestive of an increased susceptibility of these nodes to an inhibition of this reaction. Certain quantitative relations are necessary for this inhibition. It is not specific but is associated with the potency of the inhibiting filtrate as regards its capacity to elicit the Shwartzman phenomenon.

#### Discussion

(Dr. Howard T. Karsner, Cleveland.) It has been instructive to hear this interesting paper by Dr. Koplik and I wish to compliment him upon the work. Although it is difficult to interpret lantern slides, I wish to ask if the slide which he showed as illustrative of a normal lymph node from the region



of a preparatory injection does not actually show infiltration of leukocytes. The interpretation of inhibition of reaction in the lymph nodes involves determination of the reaction quantitatively. The reaction consists of various components, such as hyperemia, edema, thrombosis, hemorrhage, necrosis and infiltration by leukocytes. In order to express the reaction quantitatively it seems necessary to give each of these its own weighting, unless each were considered to be of equal significance. It would be of interest to have Dr. Koplik explain how he arrives at the quantitative evaluation of the reaction in skin, subcutaneous tissues and lymph nodes.

(Dr. Max B. Lurie, Philadelphia.) It is difficult for me to understand how the intravenous administration of the active filtrate 24 hours previous to the administration of the preparatory factor can inhibit the Shwartzman reaction when the reacting factor is later introduced intravenously. However, is it possible that Menkin's observation will explain this inhibition? He found that if trypan blue is introduced next to an area of inflammation of a certain duration the dye will not penetrate the site of inflammation. I wonder whether the introduction of the active filtrate produces the same change in the permeability of the blood vessels, so that later when the preparatory factor is introduced into the skin it will not penetrate into the blood vessel wall, thus interrupting the chain of events leading to the Shwartzman phenomenon.

(Dr. M. J. Shear, Boston.) The filtrates used contain a wide variety of substances. The histological changes might perhaps be clarified by producing the Shwartzman phenomenon with a purified agent that was separated from accompanying contaminants. It has been found possible to separate a fraction from *B. coli* filtrates which produces hemorrhage in the skin of rabbits in doses of 0.0005 of a milligram. Pathologists may find it of advantage to employ the purified agent in studies such as this.

(Dr. Koplik.) In answer to Dr. Karsner, it is perfectly true that there is a leukocytic infiltration when an intradermal injection only is given. It is also true that this infiltration is proportional to the concentration of the filtrate used intradermally. However, the amount of inflammation following preparation does not seem to bear any direct relation to the degree of hemorrhage or thrombosis that may be obtained after the subsequent intravenous injection.

With reference to a quantitative relation I think we might say from these experiments and from my previous work on the phenomenon in lymph nodes that perhaps the earliest lesion to appear is the vascular thrombosis. When the phenomenon is more marked we also get hemorrhage. Hemorrhage may be present with relatively little thrombosis, but in the present inhibition experiments the lesions in the lymph nodes which were visible only microscopically did not show hemorrhage but merely thrombosis.

As to Dr. Lurie's question regarding the permeability of the blood vessels, we have not thus far investigated the mechanism of inhibition.

In answer to Dr. Shear, the experiments on inhibition have not been done with fractions of the filtrates but only with the filtrates themselves. It seems essential that filtrates containing the Shwartzman factors themselves be employed in order to produce inhibition. Ogata failed to obtain inhibition with plain broth, peptone and streptococcus filtrates devoid of the Shwartzman factors.

THE REYNALS FACTOR IN FETAL AND ADULT GUINEA PIG TISSUES. N. Paul Hudson and (by invitation) O. N. Fellowes, Columbus, Ohio.

*Abstract.* In a study of the marked susceptibility of the guinea pig fetus to vaccinia virus (Stritar and Hudson), we have attempted to determine whether there is an extractable factor peculiar to fetal tissues capable of enhancing the action of the virus in rabbit skin. Such a principle, found by Duran-Reynals and others to be in highest concentration in the rabbit testis, has been called "the Reynals factor."

Saline extracts of fetal and adult guinea pig tissues were mixed with decreasing amounts of vaccinia virus carried in rabbit testicular tissue. The mixtures were inoculated intracutaneously and the appearance and size of lesions recorded at the end of the 6th day.

The ratios between the sizes of the virus control and test lesions were: fetal kidney 1:2.3, adult kidney 1:1.9; fetal liver 1:1.8, adult liver 1:1.7; fetal brain 1:1.9, adult brain 1:1.7; fetal skin 1:2.3, adult skin 1:1.7; fetal lung 1:1.7, adult lung 1:1.7; normal rabbit testicle 1:2.8.

Apparently there is no extractable property capable of enhancing the action of vaccinia virus, which is peculiar to the tissues of guinea pig fetuses. The transmissible factor is present in fetal and adult tissues, but not to the degree had by the rabbit testis. While the Reynals factor may be significant in the greater susceptibility of the fetus to vaccinia virus and be outweighed by some other factor, it does not appear to be the sole demonstrable basis for susceptibility.

### *Discussion*

(Dr. A. E. Casey, St. Louis.) I should like to ask Dr. Hudson whether the term "Reynals-McClearn factor" would be equally acceptable? McClearn in England reported at about the same time as Duran-Reynals.

(Dr. Hudson.) I should like to give expression to the fact that McClearn described some very fine studies. I hope we will be able to learn some more about this factor in its definition, but since we measure it here by its action, and its chemical nature has not been described, we might still use the name given. It might be well to use the names Reynals and McClearn together.

THE DEVELOPMENT OF THE LOCAL CELLULAR REACTION TO TUBERCULIN IN SENSITIZED CALVES. William H. Feldman, Rochester, Minn., and (by invitation) C. P. Fitch, St. Paul, Minn.

*Abstract.* Using a group of sensitized calves a study was made of the cellular reaction that follows the intracutaneous injection of tuberculin. After the tuberculin was injected biopsies were done at 6 to 8 hour intervals up to and including the 72nd hour and on the 5th, 7th, 10th and 14th days. The reactive process showed a constant selection for the perivascular and perineural tissues and during the early phases of the reaction polymorphonuclear leukocytes were numerous, while eosinophilic granulocytes and histiocytes were in the minority. There was a gradual but constant increase in the histiocytes up to the 72nd hour. This was particularly noticeable at and beyond the 30th hour. The polymorphonuclear leukocytes gradually diminished and between the 60th and 72nd hour the histiocytes predominated. Acidophilic polynuclear and

mononuclear granulocytes were usually numerous. The character of the reaction persisted practically unchanged up to the 14th day although most of the cells other than histiocytes had disappeared. The histiocytes in the tissue removed on the 14th day were richer in chromatin and many were becoming oval or fusiform in contour, suggesting fibroblasts. Other features of the process consisted of edema and endovascular changes such as venous thrombosis and endarteritis. Lymphocytes and plasma cells assumed a minor role in the reaction.

### *Discussion*

(Dr. Max B. Lurie, Philadelphia.) I should like to ask Dr. Feldman whether his study showed any evidence for the differentiation between the anaphylactic and so-called tuberculin type of reaction. Mallory and Dienes claimed that in the tuberculin reaction, if a small enough dose of tuberculin is given, the reaction is almost completely mononuclear in type from the very beginning, whereas in the anaphylactic reaction polymorphonuclears are the most predominant. I should like to ask Dr. Feldman if he gave these calves a sufficiently low dose of tuberculin to answer this question.

(Dr. Virgil H. Moon, Philadelphia.) I should like to ask whether Dr. Feldman has an explanation of the mechanism by which edema develops in these reactions. Edema is, of course, a feature of inflammatory reactions. Does Dr. Feldman feel it has the same mechanism here as in inflammatory reaction in general?

(Dr. E. R. Long, Philadelphia.) Apropos of Dr. Lurie's remarks, Dr. Vorwald, Dr. Holley and I in experiments in Chicago obtained results that conform entirely to those Dr. Feldman just reported. We have not seen the striking difference between the anaphylactic type of response and the tuberculin type of response that Mallory and Dienes reported. On the contrary, we have seen exactly what Dr. Feldman reported, a polymorphonuclear reaction beginning in a few hours and lasting up to 2 days, followed by replacement of the polymorphonuclear cells by mononuclears. I have thought that some difference in the inoculation dose might have produced the different results. I think what Dr. Feldman reported holds for small doses.

(Dr. Feldman.) We have not attempted to verify or investigate the anaphylactic reaction reported on by others. Our dosage of tuberculin was that usually used by the Bureau of Animal Industry of the U. S. Department of Agriculture. We used 0.4 cc. for each injection; the solution representing 25 per cent of old tuberculin.

As regards Dr. Moon's question, I cannot offer any explanation. The edema does not seem in any way different in these inflammatory reactions than it does elsewhere.

### EXPERIMENTAL STUDIES ON POSSIBLE MECHANISMS OF CERTAIN PREDISPOSING FACTORS TO LOBAR PNEUMONIA. W. J. Nungester and (by invitation) R. G. Klepser, Ann Arbor, Mich.

*Abstract.* It has been shown that sterilized gastric mucin or sputum introduced deep into the lower respiratory tract of the rat along with small quantities of pneumococci aided in producing lobar pneumonia. The question was raised as to whether or not certain predisposing factors to pneumonia might

"lower the resistance" by causing the aspiration of mucinous secretions from the upper respiratory tract to the lungs.

Exposure to cold or marked alcoholic intoxication are recognized as predisposing factors to pneumonia. These factors have been investigated from this point of view. Mucin and India ink were placed in the noses of rats under a light ether anesthesia. Some of the rats were intoxicated with alcohol, others were sprayed with cold water, and others held as controls. About 20 animals were included in each group.

Only microscopic amounts of ink were noted in the lungs of the control animals sacrificed 1 hour after inoculation; 55 per cent of the intoxicated animals and 53 per cent of the rats exposed to cold had gross amounts of ink in the lungs.  $10^{-4}$  cc. of a type I pneumococcus culture were substituted for ink in an analogous experiment. From 24 to 47 animals were included in each group. Thirteen per cent of the control animals developed lobar pneumonia. In the intoxicated group 38 per cent were found to have pneumonia, while 42 per cent of the rats exposed to cold water developed lobar pneumonia. Cold and alcohol appear to interfere with the function of the epiglottis. Aspiration of large amounts of culture without mucin did not result in pneumonia in any of 42 rats.

### Discussion

(Dr. Francis G. Blake, New Haven.) I do not think anyone would take exception to the view that the introduction of mucin or other substances of that type may predispose in certain animals to the development of pneumonia. What bearing this may have on the development of pneumonia in man, is, of course, difficult to say. In this connection it should be recalled that 100 per cent of Filipino monkeys develop typical lobar pneumonia following intratracheal injection of 0.000001 cc. of *Pneumococcus* Type I culture without mucin or other added factors.

(Dr. Nungester.) We would not want to draw any conclusions on the pathogenesis of pneumonia in man. Mucin in the tract is not absolutely necessary because we can produce pneumonia by large doses of pneumococci injected without mucin, but in a rather low incidence of cases. We believe other factors may favor the development of pneumonia in the lungs. Two such factors we have investigated are serum and plasma. Both of these when introduced into the lung along with pneumococci give a higher incidence of pneumonia than when pneumococci are introduced with 0.85 per cent saline.

THE RESULTS OF INTRATRACHEAL INJECTION OF BORDET-GENGOU BACILLI IN MONKEYS AND RABBITS. D. H. Sprunt and (by invitation) D. S. Martin and Sara McDearman, Durham, N. C.

*Abstract.* Recently several workers have reported the production of a lymphocytosis and a paroxysmal cough or whoop in apes by the injection of large numbers of the Bordet-Gengou bacilli. As we had previously reported interstitial mononuclear pneumonia resulting from the injection of the Bordet-Gengou bacillus in rabbits, we thought a further study of the morbid anatomical and the blood changes in both the monkey and the rabbit would be of interest.

Six *Cebus* monkeys and a number of rabbits were inoculated intratracheally with virulent Bordet-Gengou bacilli. Five of the monkeys and all of the

rabbits had a marked lymphocytosis and an interstitial mononuclear pneumonia. The lymphocytosis was shown to be significant as it was more than double the maximum reached in 91 counts on normal monkeys.

The Bordet-Gengou bacillus could be recovered by daily nasal cultures from any of the animals but was cultured at autopsy from only 1 monkey.

These observations indicate that both the interstitial mononuclear pneumonia and the lymphocytosis are the result of a toxin liberated by the Bordet-Gengou bacillus in the tissues and agree with the idea that the cough is the result of the multiplication of the bacilli on the cilia. A reasonable explanation of the pathogenesis of pertussis in apes is that a number of the bacilli disintegrate and produce an interstitial mononuclear pneumonia which serves as suitable substratum for the organisms to multiply and cause the whoop.

The enormous dose of Bordet-Gengou bacilli required to produce the described change suggests that another agent may be required to initiate the Bordet-Gengou infection in man; as viruses produce such a reaction it may be, as has been suggested by other workers, that infection with the Bordet-Gengou bacilli is dependent on a preceding virus reaction in the lungs.

### *Discussion*

(Dr. A. E. Casey, St. Louis.) I wonder whether Dr. Sprunt differentiated between monocytes and lymphocytes. In most diseases it is the monocyte that rises in the first week or 10 days, and the lymphocyte only after some weeks.

(Dr. Howard A. McCordock, St. Louis.) These experiments and those that preceded them from the same laboratory are of great interest to those who have concerned themselves with the peculiar pneumonias that follow certain acute infectious diseases. I am glad that Dr. Sprunt said that the pneumonia following pertussis is slightly different from the experimental lung lesions produced by massive doses of bacteria. Any adequate explanation of this problem must account, not only for the interstitial reaction which is the subject of this paper, but must also take into consideration the hemorrhagic pneumonia that often precedes it, as well as the subsequent complications such as lung abscess and empyema, all of which are associated with the infectious diseases in which interstitial pneumonia is commonly found. The acute, fulminating hemorrhagic and edematous pneumonia that occurs in those individuals who die within a few days after the onset of symptoms of epidemic influenza, is as much a part of the problem as the later interstitial pneumonia. It has been shown experimentally that one can produce all these lesions either with a virus alone or with a virus in combination with some bacteria. That is more than can be accomplished with bacterial organisms acting by themselves.

In our first publication on virus pneumonia we pointed out that many bacterial, as well as substances other than viruses, may produce an interstitial reaction in the lung. In spite of this fact we regarded interstitial bronchopneumonia as a fairly reliable histological index of virus action and I still believe it is. An acute inflammatory exudate is a useful index of the presence of pyogenic bacteria in tissue, even though it may also be called forth by sterile necrotic tissue or chemical irritants. Tubercles are produced by many foreign bodies other than the tubercle bacillus, and yet tubercles are helpful in the diagnosis of tuberculosis. Similarly it would be most unusual for an

interstitial reaction in the lung to be reserved exclusively as a defense against viruses. To infer that interstitial bronchopneumonia is useless as an index of virus action because wholesale doses of bacteria of low virulence also produce an interstitial reaction is to ignore all the other aspects of the problem.

Several investigations have shown experimentally that *B. influenzae* may produce an interstitial reaction and that large quantities of fluid cultures containing toxin may call forth a hemorrhagic and edematous pneumonia. However, interstitial bronchopneumonia is seen only in epidemic influenza, with which a virus is associated, and never in other pulmonary conditions in which *B. influenzae* may be recovered from the lung tissue.

No one from our laboratory has ever claimed that whooping cough was caused by a filterable virus and that the Bordet bacillus may be ignored in considering the etiology of this disease. We are interested in the high incidence of intranuclear inclusions in the lungs in pertussis and are seeking the explanation of their presence. To date, we have demonstrated them both in our St. Louis material and also in sections from other cities, in about 30 per cent of all cases of pertussis so far examined. They cannot be produced by bacteria. This phase of the pertussis problem can never be solved by repeating experiments with the hemophilic bacilli.

The coughing in pertussis can hardly be attributed to the sticking of bacilli between the cilia of the bronchial epithelium. This is also seen in a variety of other conditions. Influenza bacilli can be demonstrated in a variety of lung lesions in the same situation and yet these individuals have no paroxysms of coughing. It has always seemed to me that the presence of lesions in ganglia at the root of the lung explains the paroxysmal character of the cough better than the mechanical irritation of the cilia of the bronchial mucosa.

(Dr. Sprunt.) In answer to Dr. Casey, we included no monocytes in the lymphocyte counts.

I cannot, at this time, answer any of the points raised by Dr. McCordock except to point out that although a virus may cause an interstitial mononuclear pneumonia, there are also a number of other agents that may cause this same type of reaction. We have previously shown that bacterial toxins like viruses when used in large amounts cause a hemorrhagic pneumonia and when used in smaller amounts cause an interstitial mononuclear pneumonia. In closing I would emphasize that the presence of an interstitial mononuclear pneumonia does not justify definite conclusions as to the nature of its incitant.

**THE CHEMOTROPIC ATTRACTION OF LEUKOCYTES BY FRACTIONS OF *Streptococcus haemolyticus*.** H. M. Dixon (by invitation), Morton McCutcheon, and (by invitation) E. J. Czarnetzky, Philadelphia, Pa.

*Abstract.* From experiments reported before this Society last year, it was concluded that the chemotropic attraction of polymorphonuclear leukocytes to bacteria *in vitro* is brought about by substances given off by the bacteria. The present experiments represent an initial step in identifying such substances, by testing the chemotropic effect *in vitro* of certain fractions of *Streptococcus haemolyticus*.\* These fractions were: (1) a labile antigen con-

\* For the method of preparation of these fractions see Mudd, S., Czarnetzky, E. J., Pettit, H., and Lackman, D. Labile bacterial antigens and methods for their preparation and preservation. *Proc. Amer. Philos. Soc.*, in press.

sisting of a protein-carbohydrate complex, which, on injection into rabbits, produces specific agglutinating and phagocytosis-promoting antibodies; (2) a protein-free, non-antigenic, crystalline, stable hemolysin; and (3) a carbohydrate (fairly pure and freed of protein). Each of these three substances was adsorbed on kaolin (which by itself does not attract leukocytes) and was then tested as a source of attraction of rabbit polymorphonuclear leukocytes *in vitro*. The leukocytes were obtained by injecting physiological saline solution into the peritoneal cavity; the exudate was mixed with plasma and a drop of the mixture was allowed to spread between slide and coverslip, with the coated kaolin particles in the center. Under the microscope it was observed that leukocytes were strongly attracted to the particles coated with the labile antigen, but not to those coated with the other two fractions. It is concluded that of the three fractions of hemolytic streptococcus tested, the one that calls forth phagocytosis-promoting antibodies is also the substance that attracts leukocytes to the bacteria, thus making possible their phagocytosis.

**BIOLOGY OF THE INFECTIOUS AGENT OF TRACHOMA.** Louis A. Julianelle, St. Louis, Mo.

*Abstract.* In pursuing the tentative concept that the infectious agent of trachoma may be a virus, studies have been conducted on its nature as determined by its behavior under different conditions. Since experimental trachoma may be induced in monkeys with material free from cultivable bacteria, as may be demonstrated by rabbit testicular passage of trachomatous tissues, a concerted effort has been made to cultivate the infectious agent in tissue culture. For this purpose a number of technics have been employed, including tissue cultures of 6 animal species (chicken, mouse, guinea pig, rabbit, monkey and man), without, however, demonstrating propagation. Filtration of trachomatous tissue through Berkefeld V, collodion membrane (a.p.d. ca.  $0.6\mu$ ), Seitz and plaster of Paris (Kramer) filters indicates that, regardless of the method used, the infectious agent is so rarely filterable as to make this method of study impracticable. The influence of various agents, physical and chemical, reflects the extremely delicate nature of the infectious agent.

Studies on immunity to trachoma reveal that no active immunity is demonstrable following recovery from experimental infection. So, also, it was not possible to detect protective antibodies in the blood of patients or animals experimentally infected.

**LOCAL PRODUCTION OF ANTIBODIES IN VACCINIA.** O. N. Fellowes (by invitation) and N. Paul Hudson, Columbus, Ohio.

*Abstract.* Considerable attention has been given the problem of local production of bacterial antibodies (Cannon and associates), but little has been done in connection with viruses (Holden and Strong, McMasters and Kidd). We have investigated the production of so-called neutralizing antibodies in the skin of rabbits inoculated dermally with vaccinia virus.

Extracts of the skin lesion areas were made by the process of freezing and thawing. Twenty per cent extracts were mixed with diminishing quantities of virus carried free of bacteria in the rabbit testicle. Antibodies were measured by the prevention of cutaneous lesions in the rabbit in terms of the

amount of virus neutralized. The tests were conducted on skin areas bearing lesions from 1 to 30 days old. Parallel control examinations were made with the serum of a normal rabbit, a known positive serum from a convalescent rabbit, the serum from the animal furnishing the skin lesions, and extracts of normal rabbit skin and of the uninoculated skin of the rabbit having the skin lesions.

Neutralizing antibodies first appeared in the serum on the 3rd day after inoculation, mounted to the level of the convalescent serum control on the 12th day, and dropped in titer from the 16th day. Extractable antibodies were demonstrable in the skin lesion areas on the 4th day, increased in titer to the 20th day and persisted throughout the experimental period of 30 days. Antibodies appeared in uninoculated skin on the 9th day, but were not tested for after the 12th day. Normal serum and extracts of normal skin did not neutralize the virus.

### *Discussion*

(Dr. George Hartley, Jr., Chicago.) In connection with this interesting work I should like to present some evidence on the local antibody formation of antivaccinial antibodies in rabbit skin that agrees in general with the above. To obtain satisfactory proof of the local production of antibacterial antibodies it has been found necessary in this laboratory to first irritate the local area, either specifically or non-specifically. This irritation calls forth, along with other cells of inflammation, an infiltration of macrophages. Since these cells are important factors in the production of antibacterial antibodies, it was assumed that they are probably also necessary for the production of antiviral antibodies. Accordingly we first irritated the skin with a single intradermal inoculation of  $\text{Al}(\text{OH})_3$  gel prepared by Willstaater's, Type C formula for enzyme purification. This irritant has a three-fold advantage. In the first place, it is an excellent macrophage mobilizer; great numbers infiltrate the area and phagocytose the gel by the 4th or 5th day following inoculation. Secondly, the vaccinia virus is readily adsorbed to and is not inactivated by the  $\text{Al}(\text{OH})_3$  gel, thus preventing to a great extent virus generalization. And thirdly, the lesion becomes quite avascular after 2 to 3 weeks, also decreasing the chance of virus generalization.

Some 18 to 21 days following the  $\text{Al}(\text{OH})_3$  inoculation a small quantity of virus, ranging from 0.5 to 1.5 skin infecting doses in 0.04 cc. amounts is adsorbed to  $\text{Al}(\text{OH})_3$  and inoculated directly into the nodule. Three and one-half to 4 days later the animal is sacrificed and the virus inoculated nodule, together with a normal control nodule of skin, blood, spleen, liver and bone marrow, is removed, extracted and filtered through Seitz asbestos pads to remove all traces of virus that might be present in the tissues.

The results of these experiments show that 10 per cent extracts in saline of the local virus inoculated area contain in  $3\frac{1}{2}$  to 4 days approximately three times as many neutralizing antibodies as the whole blood serum and control skin area, twice as many as the spleen, and ten times as many as the liver and bone marrow. In about one-third of the animals the whole blood serum contained no demonstrable neutralizing properties whatsoever. In all cases but 1 the local virus inoculated area contained as high or higher concentration of virucidal antibodies as did the whole blood serum. The series includes approximately 25 animals. We believe, therefore, that when vaccinia



virus is adsorbed to  $\text{Al}(\text{OH})_3$  gel and inoculated into a locally prepared macrophage area of skin, which also contains  $\text{Al}(\text{OH})_3$ , most of the virus is held locally and after about 4 days the macrophages have produced demonstrable neutralizing antibodies which have not as yet, in most cases, been poured out into the blood stream.

(Dr. Max B. Lurie, Philadelphia.) A somewhat similar phenomenon is observed in tuberculosis and, to my mind, it appears that the same principle is involved. If tubercle bacilli are injected into a local area subcutaneously, it is well known that they multiply there and that some are disseminated throughout the body. When immunity sets in, the destruction of the bacilli takes place first and to a marked degree at the point of injection. Some destruction, and to a lesser degree, takes place at the same time in the draining lymph nodes immediately bordering on the lesion. Yet at this time multiplication goes on uninterrupted in the internal organs. While this is no evidence of local antibody production, it is evidence of greater immunity being produced at the point of injection of the organism and of less immunity being present in other parts of the body, and that this immunity decreases in proportion to the distance removed from the point of the first introduction of the bacillus.

(Dr. Paul R. Cannon, Chicago.) We have been hampered in the demonstration of the production of antibacterial antibodies by the complicating factor of non-specific flocculation. Last year when Dr. McMaster reported on the local formation of antibodies to vaccinia in lymph nodes, we thought that a neutralizing method might get around the difficulty of non-specific flocculation.

Dr. Walsh and I have been studying the problem of local formation of antibacterial antibodies in the respiratory tract. Our general method is as follows: We immunize the same animal locally in the respiratory tract with *B. typhosus* and intraperitoneally with *B. paratyphosus B*. We sacrifice the animals at varying intervals and titrate the tissue extracts and blood serum against both antigens. In this way we can control the non-specific flocculating factor as well as obtain a ratio of titers of tissue to blood serum for both antibodies. We have found in some instances that the titer of blood serum was identical for both *B. typhosus* and *B. paratyphosus B*. The ratio of antibody titer of respiratory tissue to blood serum, however, might be 1:1 with respect to the bacteria used for local immunization, whereas it was 1:8 for the microorganism used in the general immunization. Such an unequal concentration of antibodies in the same tissue extract, with the concentration in the blood serum being equal, is strong evidence for the local formation of antibodies as a result of local antigenic stimulation.

THE ACTION OF IMMUNE SERUM UPON INFLUENZA VIRUS *In Vitro*. T. P. Magill (by invitation) and Thomas Francis, Jr., New York City.

*Abstract.* Studies have been conducted on the effect of immune serum upon a strain of human influenza virus (PR8) grown in chick embryo tissue culture medium. The results show (1) that when cells are exposed to the action of immune serum of low titer and subsequently washed, they support the growth of virus as well as cells treated with normal serum; (2) that, in agreement with the results of other workers, when virus is added to cell sus-

pensions before the addition of immune serum of low titer, virus survives in the cells; and (3) when mixtures of immune serum of low titer and virus are added to cells there is little evidence of survival or multiplication of the virus. Furthermore, when immune serum of high titer is used the virus is inactivated irrespective of whether the cells are first exposed to virus or immune serum. Finally, virus mixed with strong immune serum is inactivated in the absence of cells as shown by the fact that centrifugation at high speeds of such serum-virus mixtures yields no active virus, whereas normal serum-virus mixtures yield fully active virus.

A CANINE ENCEPHALITIS WITH SOME SPECIFIC CHARACTERS. Robert G. Green, Minneapolis, Minn.

*Abstract.* A beagle hound about 5 months old suddenly exhibited great excitement and fear, ran about wildly, and finally fell over unconscious. A few days later a similar attack was followed by marked choreiform movements. The animal was not gravely ill when etherized and killed. Microscopic examination of tissues showed principally large acidophilic inclusions in the nuclei of the smaller nerve cells of the brain. A few affected cells occurred throughout the brain, but definite foci of inclusions were found in the cerebellum. The typical inclusion is a large uniform body, occupying about half the nuclear space and separated from the nuclear wall by a clear zone. A fine margination of chromatin is typical, but occasionally the chromatin is attached to the nuclear wall in large masses. Attempts to transmit the infection by brain emulsion to both dogs and foxes have given negative results. The large number of nerve cells involved indicates that the pathological change observed was the basis of the symptoms produced, and the typical appearance of the intranuclear inclusions suggests a virus origin of the disease.

*Discussion*

(Dr. Howard A. McCordock, St. Louis.) I should like to inquire if you examined the salivary glands of this dog. The inclusions morphologically are very similar to salivary gland inclusions. I have seen Dr. Cowdry's sections showing inclusions in the tissues of dogs that were examined for another purpose. We have seen similar inclusions in the brain of a child who apparently developed a dissemination of the salivary gland virus with the production of intranuclear inclusions in the cells of many organs.

We have also recorded the experimental production of generalized visceral lesions with mouse salivary gland virus. Knowing that the salivary gland virus occasionally becomes generalized, would it not be well to consider this possibility provided the dog's salivary gland contained inclusions?

(Dr. Green.) There were, of course, routine examinations made of the salivary glands. We are well acquainted with the salivary gland inclusions from our work in examining a large variety of wild animals. There would seem to be no species of animal in which they are not found, and in some they appear to be very common. We have not attempted to carry out studies other than to note their presence. Later we shall publish these observations. From my experience in the study of encephalitis it is my opinion that the damage done to the nerve cells in these animals is sufficient to account for the symptoms.

SEASONAL VARIATION IN THE INTENSITY OF THE BRAIN REACTION OF THE ST. LOUIS ENCEPHALITIS IN MICE AND OF ENDEMIC TYPHUS IN GUINEA PIGS. R. D. Lillie, C. Armstrong (by invitation), R. E. Dyer (by invitation), and J. G. Pasternack, Washington, D. C.

*Abstract.* In the course of Armstrong's encephalitis studies a progressive decrease in the frequency of definitely positive brain reactions occurred, reaching a maximum in June and July, and followed by a sharp increase in August. The curve of positive reactions parallels the inverted curve of the mean monthly temperatures.

High virus dosages increased the positive reactions, but the seasonal variation remained evident in all dosage groups.

In simultaneous studies on the effect of environmental temperature 58 animals at 35°C. showed 50 per cent definitely positive reactions, 14 kept at 10°C. showed 100 per cent, and 35 at 23°C. showed 83 per cent.

Monthly grouping of 177 guinea pigs with endemic typhus revealed a similar seasonal variation in reaction intensity.

A consecutive series of 517 animals over a full year showed a high period from February to May, a low period from June to August, and a high period from September to January. The curves of the average numbers of lesions parallel the mean monthly temperature, charted inverted.

Animals at 33°C. show considerably lower average numbers of brain lesions than at 24 or 18°C.

The present indications are that environmental temperature exerts a direct influence on the intensity of the pathological reactions in the brain to these two viruses.

### *Discussion*

(Dr. Albert B. Sabin, New York City.) I should like to ask whether only the inflammatory reactions in the mouse brains were studied, or were nerve cell lesions also noted.

(Dr. Albert E. Casey, St. Louis.) Some years ago Dr. Wade Brown noted that the transplantability of the rabbit tumor varied directly with seasonal change in temperature. And in studying blood cells we found the same variation with regard to seasonal temperature in red cells. These changes were related to seasonal variation in resistance. I am curious to know whether anything of that sort occurred here.

(Dr. Howard A. McCordock, St. Louis.) During the epidemic in St. Louis the first sharp drop in the number of cases was coincident with the return of colder weather. At that time we thought that the epidemic had burned itself out, because most of the susceptible individuals had either died of the disease or become immunized, but in view of this work it may be that the return of cold weather had something to do with the disappearance of the disease. However, I do not believe that the excessive heat during the summer of 1933 was responsible for the appearance of the disease because hot summer weather is no novelty in St. Louis, and last year we had a much higher average temperature than in 1933 without a return of the disease.

(Dr. Lillie.) In reply to Dr. Sabin, the grading given here relates entirely to the inflammatory reaction and not to the degree of destruction.

In reference to whether we have observed changes in the blood, we have

not studied blood changes, but we are carrying on investigations of other organs and we have not had time to bring this to any conclusive result before this meeting.

Dr. McCordock's remarks about the drop in the epidemic when the cold weather came on are interesting. I have speculated on it, but I do not know.

(Dr. Sabin.) In view of the fact that only the inflammatory lesions were noted, I wonder to what extent so-called "spontaneous encephalitis" of mice, unrelated to the virus injected, might have influenced the data. Among the Rockefeller Institute mice, which are bred under careful isolation, the majority of the older animals show meningoencephalitic lesions without exhibiting any clinical signs of disease.

(Dr. Lillie.) We had a very considerable number of other mice under study at the same time and they had not received the St. Louis virus. We did not see the inflammatory lesions of the brain.

#### THE PATHOLOGY OF GRANULOMA VENEREUM. Rigney D'Aunoy and Emmerich von Haam, New Orleans, La.

*Abstract.* The pathology of granuloma inguinale has been studied in a series of 294 cases observed over 5 years at the State Charity Hospital of Louisiana at New Orleans. The typical manifestations of the disease embrace nodular lesions and serpigenous ulcerations which have a tendency to spread along the moist folds of the pudendal region, healing with the formation of atrophic scars. Atypical manifestations are produced by unexplained increased fibroblastic reaction of the host leading to hypertrophic and cicatricial keloid-like lesions which must be considered active stages of the infection. Secondary infection produces serious ulcerative necrotic lesions which may severely mutilate the infected parts and give rise to sepsis and toxemia. Histopathological study of biopsy material and tissue secured from autopsy reveals that the stage of infiltration is quickly followed by the stage of granulation during which the epithelial lining of the skin or mucous membrane is perforated by a vascular granulation tissue. Donovan organisms can be demonstrated in the infected tissue during all stages of the infection. Search for a causal agent has resulted in the isolation of an organism belonging to the Klebsiella group. Inoculation of various laboratory animals with this organism has failed to incite lesions comparable to the disease in the human, although such lesions have been reported as produced by inoculation of the material derived from human cases.

#### AN EPIDEMIOLOGICAL APPROACH TO THE CONTROL OF TRICHINOSIS. Frank B. Queen, Denver, Colo.

*Abstract.* Fifty gm. portions, or less, of the diaphragms of garbage-fed and non-garbage-fed hogs grown in the Denver area were examined by the digestion method for the presence of *Trichinella spiralis*. In garbage-fed animals 15.5 per cent of the 522 examined were found to be infected. Of 193 non-garbage-fed animals but 0.5 per cent were infected.

These figures indicate that *Trichinella* infestation in hogs and hence the transmission of the disease to man can be controlled in this locality through the compulsory sterilization of all garbage fed to hogs. Studies are needed to determine the role of rats as an indirect reservoir of the disease for man.

If this source proves important, then with rat control measures added to the compulsory sterilization of garbage, trichinosis in man can be eliminated.

### *Discussion*

(Dr. A. E. Casey, St. Louis.) Is it possible that there are factors besides the feeding of garbage, such as housing conditions and environment, which are concerned?

(Dr. Marcus W. Lyon, South Bend, Ind.) Professor H. E. Enders of Purdue told me that the examination of rats from the vicinity of slaughter houses near Lafayette showed a very high percentage of trichinosis, whereas the examination of rats living wild in fields away from slaughter houses showed a very small amount. Trichinosis is a very important subject because the hamburger stands all over the country often use a small proportion of pork mixed with the beef. In South Bend Dr. A. S. Giordano traced several cases of infestation to eating hamburger sandwiches from automobile stands along the highway. This is a very intriguing subject. The obvious way of avoiding the disease, as Dr. Queen has pointed out, is not to feed hogs anything except good food.

(Dr. E. R. Long, Philadelphia.) I might add that studies by the National Institute of Health have shown a wide prevalence of the disease in man, and the National Research Council has taken the subject under deliberation as a special problem. Two divisions of the National Research Council expect to put on a campaign in cooperation with the National Institute of Health to stimulate pathologists to go into this more thoroughly to see if figures such as Dr. Queen gave represent the present situation throughout the country. I believe the packers are very much concerned over the reports that have come out, for sales have decreased since the amount of trichinosis has been found to be so high. I wish to announce here that you will all be circularized by the Division of Medical Sciences of the National Research Council, with the request that you cooperate with the Division by submitting samples from autopsies, so that we can get a wider range of material. At present the figures from different localities vary greatly. It seems worth while to push the matter much further because the disease is evidently more widespread than used to be believed.

### **TOXOPLASMA INFECTION IN THE GUINEA PIG.** Floyd S. Markham, Columbus, Ohio.

*Abstract.* Spontaneous and experimentally induced *Toxoplasma* encephalitis in young guinea pigs is described. Parasites were also found in the kidneys of adult stock guinea pigs. The protozoa in both adult and young animals closely resemble those observed in rabbits and mice.

### *Discussion*

(Dr. R. D. Lillie, Washington, D. C.) In the course of examination of between 1500 and 2000 guinea pig brains in the past 8 or 10 years I ran across *Encephalitozoa* in 3 individual animals in which there were from 2 to 4 or 5 cysts encountered in a series of four or five transverse sections of the brain.

One of those local clumps of parasites shown on the screen in the kidney resembled *Klossiella cobayae*, a species often found in guinea pigs' kidneys. This species is said to form minute bodies in the vascular endothelium in other organs, but I hardly believe it to be related to the toxoplasmosis of the brain.

(Dr. Albert B. Sabin, New York City.) When this Society met in New York 2 years ago we demonstrated to the groups visiting the Rockefeller Institute studies on *Toxoplasma* which were isolated from a guinea pig. Proper preservation and passage at regular intervals by intracerebral injection in mice have permitted us to maintain the *Toxoplasma* in a pathogenic stage and to carry out many studies with them. There is considerable confusion in the literature concerning the nature of the *Toxoplasma*, partly because strains, as a rule, were not maintained for comparative investigations. Dr. Markham's suggestion that *Toxoplasma* may be related to the *Encephalitozoon* group of organisms, whatever their nature may be, cannot be supported by our own studies, but does point out the difficulty of making a proper diagnosis on purely morphological grounds. We are inclined to feel that widespread pathogenicity for various mammals and birds is important for the proper identification of the *Toxoplasma*. Our own strains are pathogenic for mice, guinea pigs, rabbits, *rhesus* monkeys, and newly-hatched and full-grown chickens. Our studies indicate that the *Toxoplasma* are obligate intracellular parasites which are capable of invading many types of cells, and as regards certain host-parasite relations possess many features in common with some of the filterable viruses.

*Toxoplasma* has been studied and reported upon in connection with certain human diseases on several occasions. In 1930 and 1931 Bland of London reported studies on their possible relation to human glandular fever (acute infectious mononucleosis), but the question naturally arose whether the *Toxoplasma* which were isolated had their origin in the patient's blood or in the experimental animal (rabbit). In *rhesus* monkeys we were able to show the development of protective antibodies during convalescence. By the use of the rabbit skin protection test it may perhaps prove possible to establish whether or not human infection with *Toxoplasma* exists.

(Dr. E. C. Rosenow, Rochester, Minn.) I would appreciate an expression from these experts on this matter. This type of infection is easily demonstrated by the presence of the microbe in the lesions. I should like an expression as to the ease with which it is found in animals that have marked perivascular lesions.

(Dr. Burton R. Rogers, Chicago.) I should like to ask Dr. Lillie whether he ever found hookworm larvae or adults in the brain.

(Dr. Markham.) Probably Dr. Sabin is much better qualified to answer the question with regard to the ease with which these protozoans are demonstrated. I have reported only an isolated observation.

(Dr. Sabin.) I should merely like to repeat that identification of such parasites purely on morphological grounds may be misleading. When we first isolated our strains we took some slides to Professor Tyzzer of the Harvard Medical School for identification. He too stressed the difficulty of morphological diagnosis and showed a slide of mouse kidney, sectioned 30 years ago, which contained parasites indistinguishable in appearance from our own.

PNEUMOCONIOSIS AND PULMONARY MALIGNANCY. Arthur J. Vorwald and (by invitation) John W. Karr, Saranac Lake, N. Y.

*Abstract.* The literature contains an increasing number of reports on primary carcinoma of the lung that tend to implicate inhaled dust as of etiological significance in the genesis of the newgrowth. The authors of these reports ascribe to the dust, in part at least, a carcinogenic property and point to it as one of the factors responsible for the high incidence of the tumor as encountered today. In the majority of reports, however, no inquiry has been made into the chemical and biological characteristics of the dust, and there has been no attempt to present detailed experimental and clinical evidence that the dust in question is of etiological significance and possesses carcinogenic properties. The fact that dust is a chronic irritant has been repeatedly stressed and as such has been assumed to be a predisposing factor in the development of the tumor.

A survey of pneumoconiosis cases reported in the literature, also clinical and experimental observations from the Saranac Laboratory, do not support this view. The clinical observations comprise follow-up X-ray examinations of the chests of 15,587 men from various dusty occupations. A majority of these men had had long exposures and a large proportion was over 40 years of age. Of this group, 1356, or 8.7 per cent, had demonstrable X-ray evidence of silicotic nodulation in the lungs. The incidence of primary lung carcinoma for the entire group is 0.012 per cent and for those with silicotic nodulation 0.15 per cent.

The pulmonary tissue from 179 cases of pneumoconiosis, which had developed in relation to dusty occupations in various industries throughout the country, are in the pathological museum of the Saranac Laboratory. These cases form rather a selected group, but only 3 revealed the presence of an associated primary pulmonary malignancy.

Experimental observations on different species of animals including guinea pigs, rabbits, rats, mice, chickens and cats that have inhaled dusts of varied concentration and composition for periods ranging from 1 to 3 years reveal, without exception, pulmonary foci of chronic irritation as response to the inhaled dusts. In animals with prolonged inhalation of a high concentration of silica the foci were nodular in character and made up of hyaline fibrotic tissue not unlike those in clinical cases of silicosis. Hyperplasia of the epithelium lining the major bronchial tree was seldom observed. The terminal air passages adjacent to and within the foci of chronic irritation were often lined with a flat, compact single layer of epithelial cells. Even under these conditions, the incidence of primary lung tumor was practically nil. Only two small, well localized tumors were found in the lungs of 3338 animals that had been exposed to dust.

Our evidence is significant enough to indicate that dust inhalation seldom, if ever, stimulates a reaction that terminates in a primary pulmonary malignancy.

### *Discussion*

(Dr. Samuel R. Haythorn, Pittsburgh.) If there is any connection between the inhalation of dust, smoke and primary tumors of the lung, it probably is in connection with the tar content of soft coal smoke. With this idea in mind

we placed mice in soft coal chambers in an experiment that I am going to describe later in the day. So far we have been very fortunate, for we have had only one lung tumor, and it occurred in a mouse in the control cage where it had breathed nothing but washed filtered air. There was no chance to draw the conclusion that it was other than accidental.

(Dr. Norbert Enzer, Milwaukee.) I should like to emphasize one point made by Dr. Vorwald with reference to the presence or absence of epithelial hyperplasia in the bronchi, for that would be an indication of the degree of reaction of the epithelium to the irritant. In 125 postmortem examinations of individuals with varying degrees of silicosis the bronchial mucosa failed to show any evidence of polypoid formation or atypical papillary formation, and in only one instance was there a finding of squamous cell metaplasia, and that occurred in a tuberculous bronchiectatic cavity. In the entire 125 cases not a single instance of bronchiogenic carcinoma was noted.

(Dr. H. Gideon Wells, Chicago.) A great deal depends not only on the irritant but on what is being irritated. Dr. Slye has one strain of mice in which almost 100 per cent have developed lung tumors merely by breathing the pure air of Chicago, whereas many thousands in other strains have gone on and died of old age with no member of those strains ever showing anything of the sort, so that we always have to consider two factors. That is the difficulty in all such experimental work, unless you know the nature of the animal with which you are working.

(Dr. M. J. Shear, Boston.) I do not know whether the air in Chicago is any purer than that in New York, but the dust in the air in New York has a significant amount of tar in it. Dust from the air in New York has been collected and has been found to contain a carbon disulfide soluble fraction; this air dust tar is being tested in our laboratory for carcinogenic activity, using albino mice.

(Dr. Wells.) Probably there is more here because we use a greater percentage of soft coal.

(Dr. Vorwald.) We realize that tar has carcinogenic properties. A good many animals have been subjected to soft coal dust. Some have even lived in the soft coal mines where the dust was generated. These were included in the report.

#### STUDIES IN CARCINOGENESIS. V. METHYL DERIVATIVES OF 1,2-BENZANTHRACENE. M. J. Shear (by invitation), Boston, Mass.

*Abstract.* Previous collaborative studies showed that cholanthrene had about the same carcinogenic potency as methylcholanthrene and that therefore the methyl group of methylcholanthrene is not essential for high activity. It is now found that the 5-membered ring characteristic of the cholanthrenes is not required for high potency.

Methyl derivatives of 1,2-benzanthracene, synthesized by Prof. L. F. Fieser and his collaborators, were injected subcutaneously into Strain A mice. With 5,10-dimethyl-1,2-benzanthracene, ulceration began after 2 weeks and became extensive during the 2nd month. In 2½ months tumors were noted in 7 of 36 mice; in 4 months 20 tumors were obtained. One of these tumors was discontinued after being transplanted twice; another tumor is now in its fourth generation.



While the 5-methyl derivative produced no tumors in 20 mice in 2 months, the 10-methyl derivative produced 8 tumors in 20 mice in the same period. The former compound gave a total of only 6 tumors in 5 months, whereas the latter produced 15 tumors in 4 months. Three of the tumors produced by the 10-methyl compound were successfully transplanted; 2 of them are now in their fourth generation. The 10-methyl compound produced extensive ulceration after 1 month.

The 7-methyl, the 9-methyl, and the 5,9-dimethyl derivatives of 1,2-benzanthracene are also being tested. Of these, only the last one has displayed biological activity during the first few months.

#### EXPERIMENTAL PRODUCTION OF ANILINE TUMORS OF THE BLADDER IN DOGS.

W. C. Hueper and (by invitation) H. D. Wolfe, Wilmington, Del.

*Abstract.* The investigation was undertaken to obtain experimental proof for the existence of causative interrelations between prolonged exposure to certain aromatic amines and the development of tumors in the urinary bladder. Sixteen female dogs were treated since May, 1935, with daily subcutaneous injections of 12 to 15 mg. of commercial betanaphthylamine. As there was no evidence of tumor formation in the bladder after 1 year of continued treatment 10 dogs of this series received in addition to the injections daily 150 to 450 mg. of betanaphthylamine by mouth. In January, 1937, 2 dogs of the oral group showed diffuse papillomatosis of the urinary bladder on cystoscopic examination. Two months later single papillomas were found in the bladders of 2 additional dogs, while a 5th dog had a suspicious lesion. The tumors were mainly located in the dome and the anterior wall of the bladder, that is, in the dependent parts. Biopsies taken through the cystoscope showed on microscopic examination papillary lesions of benign and malignant character resembling closely, in regard to the morphology of the tumor parenchyma and stroma, those occurring in men after exposure to aromatic amines. One dog died 3 days after cystectomy and uretero-utero-anastomosis and had in addition to numerous sessile and pedunculated neoplasms of the bladder a marked nodular cirrhosis of the liver. Lactating breasts were present in 7 of the 16 dogs on external examination in March, 1937.

#### Discussion

(Dr. Stanley Reimann, Philadelphia.) I should like to know what the 2 per cent of impurities are.

(Dr. Virgil H. Moon, Philadelphia.) What was the character and distribution of the cirrhotic changes seen in the liver?

(Dr. Hueper.) The betanaphthylamine is 98 per cent pure and contains almost 1 per cent of beta-betadinaphthylamine, which is structurally very closely related to the 3,4,5,6-dibenzcarbazone, which according to Cook produces epitheliomas of the skin of the mouse. We do not know at the present time whether massive doses of betanaphthylamine produce that effect, or whether a metabolite of betanaphthylamine, according to Cook, 3,4,5,6-dibenzcarbazone, or an impurity in the betanaphthylamine, such as beta-betadinaphthylamine, is the carcinogenic agent.

So far as the distribution of the cirrhotic changes goes, the cirrhotic changes

were most marked in the right liver lobes, and they appeared as indistinctly outlined white-brown nodules projecting above the surface. Histologically they appeared as rather diffuse proliferations of liver cells, in places invading degenerative parts of the original liver structure.

Histological examination of the breast showed a typical lactating breast.

#### CARCINOMA IN FROGS AND THE PROBABLE ETIOLOGICAL RELATION TO A VIRUS.

Balduin Lucké, Philadelphia, Pa.

**Abstract.** For the experimental study of cancer, warm-blooded animals such as rodents and fowls have hitherto been used. Recently it has been shown that a cold-blooded and more primitive animal, the leopard frog (*Rana pipiens*) is commonly affected with a carcinoma, and that this tumor is suitable for experimental studies (*Am. J. Cancer*, 1934, 20, 352, and 1934, 22, 326). It is a typical neoplasm, and it has the particular interest in that its cell nuclei frequently contain acidophilic inclusion bodies of a type characteristic of virus activity. Four hundred and twenty-two cases of the tumor have been examined; of these 364 are spontaneous growths and 58 are from frogs inoculated with tumor material.

In the present paper further observations on metastasis are recorded, and evidence is presented that the frog carcinoma is probably caused by a virus.

**Metastasis:** In our earlier studies but few examples of dissemination (3 among 276 cases of tumor) were encountered; this apparent rarity of metastasis seemed to be a fundamental difference between the frog carcinoma and structurally similar tumors of man and other warm-blooded animals. That no such difference exists is proved by our more recent findings of 17 cases of neoplastic dissemination among the 146 frog tumors examined since the publication of the previous report. Commonly, the metastatic tumors are multiple and involve more than one organ, the liver being one most often the site of the secondary tumors. Tumor emboli lying within intrahepatic branches of the renal portal veins in several of these cases suggest not only the route of spread, but make it certain that we are dealing with true cellular metastasis and not with secondary growths incited by dissemination of a causal agent. The pancreas, lung, various parts of the gut tract and the orbit are other sites to which the tumor has extended, also, in all probability, by way of the blood. On the other hand, some secondary tumors lying in the urinary bladder and the ovarian stroma may represent direct implantation of fragments detached from the primary growth rather than transport by the bloodstream.

**Transmission Experiments:** Material from 45 different tumors has been inoculated by various routes (intramuscularly, into the lymph sacs, the pleuro-peritoneal cavity, and intracranially) into a total of over 800 frogs of the same species and from the same general locality as the tumor-bearing frogs. A somewhat larger series has been kept under identical conditions as controls. In 34 of the experimental groups living tumor fragments or cell suspensions were used; in 10 tumor desiccates, and in 1 group glycerinated tumor material. The complete results are not as yet available since some of the animals are still alive, but the majority have been examined. While in some frogs the transplanted fragments persisted for several months and at the time of examination presented evidence of cell multiplication (mitoses), most fragments soon retrogressed, and no growths of significant size were observed in any of

the groups at the site of inoculation. However, a considerable number of the inoculated frogs developed tumors in the kidney. These renal tumors were morphologically identical with the spontaneous carcinomas; their incidence increased progressively with the length of period of survival after inoculation. Thus, in frogs that died or were killed within 3 months after inoculation, the incidence of the renal tumors was approximately the same as in the control groups, *i.e.* 2 to 3 per cent. In frogs that survived for from 4 to 6 months the frequency of renal tumors rose to 9 per cent, and to double this figure in the groups surviving for more than 6 months. No difference was observed between the groups that were inoculated with living material and those that had received desiccated or glycerinated tumors; in both series kidney tumors occurred with approximately the same frequency and after the same interval following inoculation. The fact that tumors developed as readily in animals inoculated with tumor desiccates as with living cells, and that these tumors developed not at the point of inoculation but in the tissue in which they naturally occur argues, we believe, for the existence of an organ-specific virus.

DEVELOPMENT OF ODONTOMAS IN RATS FOLLOWING A PROLONGED CHRONIC VITAMIN A DEFICIENCY. Caspar G. Burn and (by invitation) Alvin U. Orten and Arthur H. Smith, New Haven, Conn.

*Abstract.* A mild chronic vitamin A deficiency has been produced in albino rats by administering by mouth minimum quantities of a standardized dose of cod liver oil. The amount of vitamin A given daily was determined for each individual rat by the body weight, appearance of eyes, snuffles and vaginal smears. The average daily dose of vitamin required from 0.7 to 8.0 International Units. The most striking gross change in the rats surviving from 80 to 365 days consisted of loss in color of the incisor teeth, distortion of shape, such as twisting, transverse and longitudinal ridging, and eventual exfoliation of the erupted portion and tumor formation. Histologically some of the rats showed changes compatible with those found in a complete vitamin A deficiency. Tumor growths (odontomas) developed in over 60 per cent of the rats surviving 365 days. A few rats developed supernumerary incisor teeth. The tumor consisted chiefly of spindle shaped cells similar to the embryonic cells of the pulp tissue. Inclusions of odontoblasts and epithelial cells were distributed throughout the tumor proliferation. Imperfect forms of germinal tooth centers and osteodental structures were frequent. The molar teeth of the rats did not show tumor growths.

*Discussion*

(Dr. S. Burt Wolbach, Boston.) It is needless to say I am tremendously interested in the consequences of this very prolonged partial vitamin deficiency. From what I have seen myself I am not surprised that these results have been obtained. I doubt very much whether the majority of us here would accept the newformations as tumors in the sense in which we usually think of tumors, but nevertheless I think they may very well be. The one thought I should like to leave with you is that after all a vitamin deficiency deprives a cell of something necessary, and according to recent progress in biological chemistry it seems very probable that the removal of a vitamin

interferes with some one particular type of biochemical system. When we can do this to cells and leave the cells under conditions that enable them to survive and to multiply, we have produced very extraordinary conditions. I think this work represents a much greater progress in that line than we have made in Boston.

**EXPERIMENTAL CARCINOMA OF THE PROSTATE.** Robert A. Moore and (by invitation) Robert H. Melchionna, New York City.

*Abstract.* The paper reports a study of the effects of injection of a carcinogenic chemical, 1:2 benzpyrine, on the prostate, and the relation of the testis to the production and course of the tumors produced.

White rats of unknown genetic constitution, but derived from one breeding colony and maintained on an adequate diet, were used. The animals were about 150 days of age at the time of first procedure except those designated as senile, which were over 500 days of age. Castration was done through a single abdominal incision. The 1:2 benzpyrine in 5 per cent concentration was dissolved in lard at temperatures not above 100°C. and allowed to congeal. One-tenth of a cc. was injected through a fine needle into each anterior lobe of the prostate exposed by a midline incision. Lard alone was injected into control animals. The tissues were fixed in Bouin's fluid and paraffin sections stained with hematoxylin and eosin.

There was a total of 18 rats in which no additional procedure was introduced. These animals died or were killed from 110 to 210 days after a single injection of benzpyrine. Seventy-two per cent showed carcinoma and 5 per cent also sarcoma. In 20 animals subjected to castration at the same time that the benzpyrine was injected and autopsied 70 to 210 days afterward, the incidence of carcinoma was 65 per cent and of sarcoma also 5 per cent. Twelve senile rats were injected and observed up to 352 days after the experimental procedure. In 7 the period of observation was comparable with the above two groups and the incidence of carcinoma was 86 per cent with no example of sarcoma. The remaining 5 were autopsied after 250 to 352 days and all showed carcinoma and 2 developed sarcomas.

Six adult animals on the 178th day after injection were subjected to a laparotomy, a small biopsy specimen of the prostate taken and the testes removed. Thirty-five days later the animals were killed and the character of the tumor in the biopsy and autopsy sections compared. There was no essential difference. Four animals were castrated and injected with benzpyrine and after the 86th day given a daily injection of 0.25 mg. of the propionic acid ester of testosterone (Oreton, Schering). One animal died after 48 daily injections and the other 3 were killed after 62 injections. All showed well developed carcinoma and the latter 3 definite sarcoma. All control animals were negative.

In a study of the histogenesis of the tumors it seems probable that the action of benzpyrene is first to produce squamous metaplasia of the normal columnar epithelium and then malignant proliferation of the metaplastic cells. The sarcomas originate in the stroma immediately adjacent to a benzpyrine cyst and usually, but not exclusively, in cysts that are not lined by epithelium. It is probable that this tumor is a leiomyosarcoma.

*Discussion*

(Dr. Marion, Chicago.) Has lard alone been used as a control?

(Dr. Howard T. Karsner, Cleveland.) In order to clarify the matter I want to ask Dr. Moore two questions. One is as to whether or not sarcoma and carcinoma occur simultaneously in the same prostate, and the other is as to what evidence he can bring forward to support the contention that the sarcoma develops from smooth muscle cells.

(Dr. Moore.) I should have mentioned that we have 20 animals of the intact type and 15 castrates that were injected with lard alone. There was in some a foreign body type of reaction and slight fibrosis. There was never any evidence of metaplasia or any evidence of neoplastic proliferation of the connective tissue.

All the sarcomas we have observed have been in glands that also showed carcinoma. Sarcoma has never occurred alone.

As evidence for the smooth muscle, we have given a great deal of attention to this because in some areas there was evidence that the tumor might be derived from the skeletal muscle in front of the prostate, about the bladder neck. There was also histological evidence that it was growing from the adventitial cells about the blood vessels. We have come to the tentative conclusion that it is derived from the smooth muscle on the basis of differential stains; it stains as smooth muscle fibers with Van Gieson's, Mallory's and Masson's stains, and there was no evidence of reticulum formation in among the tumor cells.

**GYNECOMASTIA ASSOCIATED WITH INTERSTITIAL CELL TUMOR OF THE TESTIS.**

John W. Budd, Los Angeles, Calif.

*Abstract.* A 42 year old white male had suffered from enlarged and painful breasts for 5 months and impotence for 2 months. Examination revealed a swelling in the right testis which upon exploration was proved to be an encapsulated tumor 25 cm. in diameter. Microscopically the tumor cells were identified as interstitial cells. Assay of the urine and tumor tissue was negative for the presence of prolan. Twenty mouse units of estrin were found in 1.73 gm. of tumor. In the 3 months following operation there has been a material reduction in the size of the breasts and the impotence has been partially overcome.

*Discussion*

(Dr. E. T. Bell, Minneapolis.) I think there is a serious question here whether this is an interstitial cell tumor or not. Its morphology is very much like that of the ordinary embryomas of the testis, and its hormonal effect is just the opposite of what we would expect from an interstitial cell tumor. Interstitial cell tumors secrete the male hormone, and I do not see how you can connect gynecomastia with the male hormone. My impression of the tumor is that it is an ordinary embryoma of the testis.

(Dr. H. Edward MacMahon, Boston.) I should like to ask about the state of the tissue in the testis surrounding the tumor as well as the condition of the other testis. Dr. Budd told us the patient was impotent for 2 months.

Gynecomastia associated with impotence is seen in cases of fibrosis and atrophy of the testis in the absence of any tumor.

(Dr. Paul Klemperer, New York City.) I saw one tumor I thought was an interstitial cell tumor of the testis, and there was one striking feature—marked pigmentation. In the tumor presented the gross description was yellowish white. Was there any pigment visible microscopically?

(Dr. Virgil H. Cornell, New York City.) May I ask what the effect of the operation was on the gynecomastia?

(Dr. Victor Lespinasse, Chicago.) What about the hormones in the urine?

(Dr. Budd.) Last year Dr. Bell and associates reported a case of interstitial cell tumor in a boy associated with the macrogenitosomia syndrome, one of 3 cases in the literature. I think the physiological effect on an adult from the excessive hormone secretion might be nil or of a different nature. I am glad of Dr. Bell's criticism, and I should like to exchange slides with him.

Dr. MacMahon asked about the other testis. The other testis is normal by palpation; the patient has been seen several times since the operation and it still remains normal. The testis removed with the tumor showed rather marked atrophy; spermatogenesis was greatly reduced, more than the mere mechanical pressure of the tumor would account for.

Dr. Klemperer asked about the pigmentation. The color of the tumor was not so striking as in some cases of interstitial cell tumors that have been described. There was a definite yellowish color, but pigment microscopically was not found. This tissue was fixed in Zenker's, and I have noticed that Zenker's fixative does have some tendency to dilute pigmentation in the cells, and perhaps that would obscure a minor amount of pigment present.

Dr. Cornell asked about the effect of the operation on the gynecomastia. In the 3 months since the operation there has been a definite reduction in the size of the breasts. They are still larger than the breasts before the onset of illness, but there is a very definite clinical reduction in the size of the breasts.

In regard to the question about the hormones, this patient's urine previous to operation was assayed for prolan and none was demonstrated. The tumor tissue was assayed for prolan and none was demonstrated. The acetone extract of 1.73 gm. of tumor tissue revealed the presence of 20 mouse units of estrin. The hormone which we are interested in is the male sex hormone. The identity of the tumor was not appreciated until after the permanent sections were viewed, so that the matter of assaying for male sex hormones was delayed, and this is being conducted at the present time. I am sorry I do not have the reports to present at this time.

**INTRINSIC FACTORS IN THE ETIOLOGY OF NEOPLASMS.** Carl V. Weller, Ann Arbor, Mich.

*Abstract.* In the etiology of neoplasms both intrinsic and extrinsic factors have a share. The part played by intrinsic factor may be evaluated, so far as our present knowledge permits, by both clinical and experimental studies. From the clinical side the family histories of carcinoma patients, the study of families with a high incidence of carcinoma, the occurrence of multiple neoplasms in the same patient in excess of chance distribution, the modes of inheritance of particular neoplasms, and the occurrence of simultaneous and/or

symmetrical similar neoplasm in monozygotic twins have been the chief lines of approach. Each of these must receive critical evaluation. From the experimental side the breeding of strains of laboratory animals that develop spontaneous carcinomas to a high degree, or not at all, has been followed by more precise methods of investigation according to the technic of present day genetics. Into animals of tumor-producing and non-tumor-producing strains, and into their hybrid offspring, neoplasms have been transplanted and the results analyzed. Furthermore, the response of animals of varying constitution in respect to carcinoma production, and to known extrinsic carcinogenic agents reveals varying levels of resistance to such agents. From these many lines of approach it is evident that intrinsic factors enter at two levels. For the development of any neoplasm the intrinsic factor of potentiality of cellular multiplication and growth must be present. In addition there is abundant evidence that predisposition to neoplasia depends in varying degrees and through varying mechanisms for different neoplasms on intrinsic, and sometimes inheritable factors. Some of these are effective through the production of potentially preblastomatoid somatic variations. From the practical side, and for any particular human being, the importance of carcinoma in his family history must be interpreted with due regard to carcinoma type, anatomical distribution, and totality of carcinoma incidence.

#### RESPONSES TO CARCINOGENIC CHEMICALS ANTECEDENT TO TUMOR FORMATION. S. Burt Wolbach, Boston, Mass.

*Abstract.* For studies of connective tissue responses cylindrical pellets composed of 5 per cent 1,2,5,6-dibenzanthracene and 95 per cent cholesterol were introduced subcutaneously in mice.

For studies on epidermal responses mice were painted on the skin of the back with solutions of 3,4-benzpyrene in benzene. The responses in the liver to 2-amino-5-azotoluol (o-amido-azotoluol) were studied in rats and in mice. With rats, the chemical was introduced with the food (Yoshida). With mice, Shear's method of injecting the chemical suspended in glycerin into the subcutaneous tissues was used.

The effects of these three chemicals were followed histologically. All three proved to be destructive agents. With all three the sequences indicate that constant reparative processes are maintained. These reparative processes involve repair of damage to individual cells, as well as replacement of cells by division of adjacent cells. With all three agents a period of hyperplasia occurs and there is evidence that this takes place only after some degree of resistance has been acquired by the cells concerned to the agent employed. This is particularly striking in the case of the connective tissues about pellets containing dibenzanthracene. In all three experiments reparative responses on the part of resistant cells are the presumable explanation of hyperplasia, strikingly evident in islands of regeneration in the liver in the o-amido-azotoluol experiments and in the behavior of hair follicles in skin-painting experiments with benzpyrene. Even after the stage of hyperplasia is reached, evidences of the injurious effects of the chemicals on the cells continue to appear. Maintained reparative responses in regions of hyperplasia seem to be the stage preceding the development of true tumor.

No evidence was found that could be used to support a theory that any

one of the chemicals employed owes its carcinogenic property to direct stimulation of cell growth.

RELATION OF FILTERABLE AGENTS TO TUMOR FORMATION. Peyton Rous, New York City.

*Abstract.* Filterable agents are responsible for various tumors of the domestic fowl and for a skin papilloma of the rabbit, which frequently becomes carcinomatous. The renal tumors of the leopard frog are probably due to such a cause.

The agent producing the rabbit growths is characteristically a virus. Several reasons have been advanced for supposing the chicken tumor agents to be of a different sort, but with increasing knowledge it has become plain that they too fall into the virus class. The view that they are lipids is not tenable.

Under ordinary conditions cancer does not develop from the rabbit papillomas consequent on cutaneous inoculation until they have proliferated for several months. If, however, the virus is thrown into the blood stream of animals that have been tarred on the ears for a brief period, it localizes in the disordered skin and primary carcinomas arise there as well as papillomas. The way in which the malignancy comes about has still to be elucidated.

The viruses responsible for the chicken and rabbit tumors act as the immediate, directly inciting causes of neoplasms. The other "carcinogenic" agents thus far studied appear to do no more than prepare the tissue for the action of some immediate cause of nature still unknown.

SEX HORMONES AND THEIR RELATION TO TUMORS. Leo Loeb and (by invitation), E. L. Burns, V. Suntzeff, and Marian Moskop, St. Louis, Ill.

*Abstract.* An ovarian hormone, estrin, acting in cooperation with hereditary factors ( $H \times S = C$ ), causes the transformation of normal mammary gland tissue into carcinomatous tissue. This change takes place step by step and is the result of a summation of growth stimulations. In addition, the growth-inducing stimuli acting on the mammary gland during pregnancy intensify the carcinomatous transformation. Estrin induces also in vagina and cervix the formation of epithelial processes reaching into the connective tissue and progressing to the production of carcinomatous or carcinoma-like lesions in a certain number of cases. This process also takes place step by step. Estrin produces therefore carcinomatous or carcinoma-like changes in tissues in which it normally initiates specific growth processes. An increase in the amount of tissue, which occurs in response to hormonal stimulation, is not the essential or a necessary condition in the carcinomatous transformation, but it represents an associated factor produced by the same condition which is responsible for the development of carcinoma. Also, sarcoma may develop in the injected mice, but this is the result of non-specific growth stimulations.

Enlargement of the anterior pituitary gland which follows long continued estrin injections is not directly connected with the development of mammary gland carcinoma, inasmuch as this enlargement may occur most frequently in low tumor rate strains. However, it is possible to induce carcinoma formation in the mammary gland in suitable mice through multiple transplantations of anterior pituitary glands.



In high and low tumor rate strains the effects of estrin injections on the mammary glands are similar during the first 3, 4 or 5 months of injections; only subsequently the response of this tissue begins to differ in different strains. The hereditary differences in the tendency to the formation of carcinoma depend probably on differences in the mode of response of a certain tissue in different individuals or strains to long continued stimulation. The hereditary conditions underlying the proliferative changes in the mammary gland and in vagina and cervix are not the same. These differences do not depend, therefore, on differences in the rapidity of elimination of estrin from the organism.

The change of whole mammary gland tubules and acini into carcinomatous tissue, which may occur simultaneously in various parts of this organ, cannot be due to somatic mutations, nor does it depend on preceding inflammatory conditions. It is due to the cumulative action of growth stimuli of various kinds in association with hereditary factors. The action of carcinogenic hydrocarbons is in principle not different from that of other carcinogenic factors; they all seem to act through long continued growth stimulations. It may be assumed that the carcinogenic effect of hormones and other growth-inducing agents depends on an intracellular production of growth-stimulating substances which are constantly newly formed by processes similar to autocatalysis, or which are otherwise self perpetuating. It may be furthermore assumed that under certain conditions these substances are separable from the cells in which they developed and that they may then induce the production of similar growth substances in analogous tissues of other animals.

The recent experiments of Peyton Rous concerning the action of a virus in epidermal carcinoma in the rabbit suggest that possibly also in hormonal carcinomas extrinsic viruses may play a role. However, this question must be left for further investigation.

#### HISTOPATHOLOGICAL CHANGES IN MARKED SWELLING OF THE BRAIN. Kornel L. Terplan, Buffalo, N. Y.

*Abstract.* For several years systematic histological studies of the central nervous system were undertaken in cases that showed a distinct swelling of the brain postmortem. In all of these cases certain clinical symptoms such as stupor, unconsciousness, twitching, convulsions, and usually severe coma, had pointed to grave functional disorders of the brain. These studies started with a most impressive case of fatal insulin shock in a 16 year old boy with excessive swelling of the brain and the spinal cord. Histologically in this case marked structural changes in the cortex with almost complete disappearance of certain ganglion cell layers were found.

In order to get more information about the etiological and also perhaps the pathogenetic factors involved in this destructive process a large series of cases was examined, all of which showed distinct swelling of the central nervous system at autopsy.

This presentation includes the findings in the following conditions: 2 cases of insulin shock; 3 cases of acute encephalitis in children, in 1 of which severe hypoglycemia was produced by faulty administration of insulin; 1 case of oleum *Chenopodium* poisoning; 1 case of second degree burns with a past history of whooping cough; and 1 case of cerebellar cyst with diffuse swelling

of the brain. In this last instance, previous to operation, under the effect of avertin, respiration had ceased temporarily.

The histological changes found in the cortex of all of these cases suggest, in spite of the different causative factors, a similar pathogenetic mechanism. These changes, however, were not found in uremia, diabetic coma, inner hydrocephalus, purulent meningitis, and a considerable number of other conditions that were used as controls.

At postmortem examination, which, as a rule, was performed within the first 6 hours after death, the brain showed a marked swelling with distention of the dura and completely flattened gyri. Very little spinal fluid was seen within the ventricles and hardly any in the subarachnoid spaces. The weight of the brain was always distinctly increased.

The histological changes in many of these cases appeared to be of such marked intensity that they should be readily detected without any specific knowledge of the finer nervous structures. They consisted of distinct swelling of the entire cortex with marked destruction of the normal architecture. The nerve cells, especially in the third and fifth layers, were almost entirely destroyed, which made recognition of the typical layering very difficult. Where the ganglion cells could still be recognized colliquation necrosis or ischemic necrosis was present; other cells appeared as so-called ghost cells. The capillaries showed extreme injection and swelling of the endothelial cells, and the entire capillary network was conspicuous. In addition, there was slight or moderate proliferation of neuroglia cells, some of which showed severe regressive changes. These changes were especially marked in 1 case of insulin shock and in that of the cerebellar cyst where breathing had ceased temporarily under the effect of avertin. The frontal lobe, the island of Reil, and the hippocampal gyri, including Ammon's horn, were more markedly involved than the central and occipital areas. In certain instances, especially in the case of oleum *Chenopodium* poisoning, there was an absence of nerve cells, practically restricted to the third layer. Only in 1 case was it possible to study the later stages of this severe destruction. In this case the patient died 11 days after a state of complete unconsciousness from anesthetics given during an operation for fibroids. Respiration in this case had ceased for a short time. The nerve cells in the third layer were entirely replaced by a dense network of proliferating capillaries. There was also considerable demyelination in the cortex, especially in the frontal and occipital area.

Changes similar to those found in the cases of insulin shock have been produced in the last 2 years in rabbits and dogs in experimental insulin shock by Grayzel, and Stief and Tokay. Grayzel felt that the severity of the cortical changes was parallel to the number and severity of the convulsions seen in these animals. The convulsive mechanism has been stressed, particularly by Spielmeyer in his pathogenetic studies, with regard to the changes in Ammon's horn in genuine epilepsy. To my knowledge, however, such extensive changes in different cortical areas have not been observed in cases with death in acute epileptic seizures. In 2 of our cases presented here, at no time were convulsions observed. Apparently the pathogenetic factors include severe nutritional disturbances in the widest sense, such as lack of blood supply or lack of oxygen alone, or, especially in insulin shock, the incapacity of the tissues to utilize the oxygen present in normal amount in the blood. From the control material we have examined so far we do not feel that mechanical factors

alone will produce this type of cortical destruction. The findings here presented again prove the marked sensitivity of the third layer in the cerebral cortex, and of the valleys between the gyri where all the changes appeared especially intense.

### *Discussion*

(Dr. Shields Warren, Boston.) In the few cases of insulin shock which I have had an opportunity of seeing at autopsy I was rather more impressed by the degree of cerebral edema immediately beneath the ependyma in both the hemispheres and the midbrain than I was by that in the cortical layer. I wonder what Dr. Terplan's observations have been in regard to that.

(Dr. Sheldon A. Jacobson, New York City.) I should like Dr. Terplan to tell us something about these brains from the mechanical point of view. It is rather difficult to understand just how swelling of the brain as a whole could take place to a great extent. I understand in these cases there must have been a diminution in the amount of cerebrospinal fluid. In general, I think the diagnosis of edema of the brain is often made when one should say wetness of the brain. Certainly that is true in most of the brains called edematous which I have seen. Wetness might represent a change from a gel to a sol without any change in the fluid content. Schmorl made a study of the intra-vertebral discs and found that while there was an enormous difference in wetness, on chemical analysis there was no change in the water content. It is easy to understand how brain tissue might swell up when there is an abscess with destruction; otherwise it is hard to understand.

(Dr. Virgil H. Moon, Philadelphia.) I should like to ask Dr. Terplan if records were made of the visceral changes in parts other than the brain; whether congestion and edema of the lungs, the gastro-intestinal mucosa and the liver and kidneys were features in these cases. I have had no opportunity to make postmortem examinations on cases of insulin shock, either clinically or experimentally produced, but I have the feeling that changes of a circulatory character would be found similar to those that accompany shock originating otherwise.

(Dr. Terplan.) In reply to Dr. Warren, in all of the cases presented the entire brain was examined, which included, of course, the periventricular structures. Marked edema or, as I prefer to say, distinct swelling of the brain tissue, was present throughout, especially around the ventricles which, as I have mentioned, were distinctly compressed by the swollen brain. However, severe destructive lesions which were so conspicuous in the cerebral cortex were not seen in the basal ganglia around the ventricles. Only the striatum showed slight defects of nerve cells.

In regard to Dr. Jacobson's remarks, the term "swelling of the brain" was used in the sense of Reichhardt. In these cases the dura mater was markedly distended by the swollen brain, the gyri were flattened, and the sulci entirely obscured, as were the basal cisternae. There was a minimal amount of spinal fluid in the ventricles. The absence of spinal fluid in the basal cisternae, and the distention of the spinal dura by the swollen cord in the cases of insulin shock were very impressive. Of course it is mandatory to perform the postmortem examination very shortly after death to observe true swelling of the brain. We know that the amount of spinal fluid decreases about 6 hours after death by 25 per cent, and about 6 hours later by almost 50 per

cent. There exists, however, a distinct diffuse brain swelling outside of col-lateral edema around abscesses or other lesions. The term "disturbed colloid balance" has been used as an expression of true swelling of the brain by Schlueter and Seifert (London) who claim that the freezing point of the white matter in these cases is decidedly lowered. The markedly diminished amount of spinal fluid seems to me the most important diagnostic criterion for true swelling of the brain.

In answer to Dr. Moon, the liver in the cases of insulin shock was carefully examined as we were especially interested in the glycogen findings. Glycogen was entirely absent, and neither grossly nor microscopically were changes of distinct edema noted. In the lungs especially, only recent petechial hemorrhages in the pleurae and slight hypostasis were seen, but I do not recall any impressive edema in the viscera outside of the brain.

A PRACTICAL CLASSIFICATION FOR HEPATIC CIRRHOSIS. Virgil H. Moon, Philadelphia, Pa.

*Abstract.* Cirrhosis is best defined as *chronic diffuse hepatitis*. An etiological classification is impracticable, for the causes of hepatitis are various and some are obscure. Different cirrhotogenic agents may produce the same type of disease. Hence, a satisfactory classification must be based on morphological characteristics, *i.e.* on the nature and distribution of the process.

Morphological studies on portal cirrhosis indicate that its distinguishing feature is destruction of lobular pattern. There are no longer *lobules* but only *nodules* of hepatic cells. This criterion has been used satisfactorily in making differentiations. The following classification is based on it.

#### CHRONIC HEPATITIS — CIRRHOSIS

##### 1. With Obliteration of Lobular Pattern

- (a) Portal cirrhosis (Laënnec's)
- (b) With extensive pigmentation — hemochromatosis

##### 2. Without Obliteration of Lobular Pattern

- (a) With perilobular fibrosis  
Perilobular (monolobular) cirrhosis
- (b) With biliary obstruction  
Biliary (obstructive) cirrhosis
- (c) With fibrosis about central veins  
Central cirrhosis (chronic passive congestion)
- (d) With irregular varying fibrosis
  - (1) With evidence of active degeneration and repair  
Early portal (Hanot's, hypertrophic)
  - (2) Fibrosis of irregular distribution  
Atypical cirrhosis

#### Discussion

(Dr. Paul Klemperer, New York City.) Dr. Moon, if I understood you correctly, you would not make any difference in your classification between the so-called toxic cirrhosis and the complex group of Laënnec's cirrhosis.

I wonder if I am correct in that, because I do feel that we should make a very definite distinction between the so-called toxic cirrhosis, which is primarily due to a destruction of the parenchyma and leaves the framework intact, and Laënnec's cirrhosis. Comparing the anatomical and histological picture in toxic cirrhosis with that in Laënnec's cirrhosis, in which I realize there is also parenchymal destruction, I believe the parenchymal destruction is not primary in Laënnec's cirrhosis. I wonder if we should define toxic cirrhosis as diffuse chronic hepatitis; the inflammatory factor in toxic cirrhosis is secondary and not primary. In regard to the so-called Hanot's cirrhosis, I think Hanot had something very definite in mind; certainly one group of his cases was an intrahepatic biliary obstructive cirrhosis. It is the obstruction of the intrahepatic bile ducts which leads to a biliary obstructive cirrhosis.

(Dr. Moon.) In answer to Dr. Klemperer's question, this classification is based on morphology, rather than on etiology. The term "toxic" implies an etiological agent. There are several kinds of cirrhosis which can be produced by toxic agents, and that is one reason for confusion in classification. I should prefer to speak of a portal cirrhosis due to arsenicals, a portal cirrhosis due to streptococcus infection, a portal cirrhosis due to carbon tetrachloride, and so on, when the etiological agent is known.

Regarding that group of cases classed as atypical, I recall that our esteemed colleague, Dr. Mallory, surveyed 10,000 autopsies and found 590 cases of cirrhosis. Of these he found that 18.5 per cent were not classifiable. Cirrheses having the irregular type of distribution illustrated in the last two slides do not conform to any classification, nor do they produce characteristic symptomatology. The only types that produce definite symptoms are the portal cirrhosis, biliary cirrhosis with obstruction, and perhaps Hanot's cirrhosis.

Regarding Hanot's cirrhosis, the first International Congress for Geographic Pathology had as its subject Cirrhosis. Hanot's was one of the types considered. Reports were presented from 26 countries. It was interesting to note that from France, the country of Hanot's origin, there was only 1 case reported. There was not 1 case from the United States. Hanot's cirrhosis is a conception that has no definable entity. I prefer to classify cirrheses that are not characteristic morphologically as *atypical cirrhosis*.

THE INTERRELATION OF OSTEOGENIC TUMORS. Sheldon A. Jacobson (by invitation), New York City.

*Abstract.* An attempt is made to unify our concepts of the tumors of bone, and particularly of those that are not self-limited. The solitary osteomas and osteochondromas represent, of course, the benign form. It has been pointed out that the level of physiological activity of bone varies from its height in the metaphyses through the epiphyses and round (carpal and tarsal) bones to its lowest point in the diaphyses. This is exemplified by their differential growth rates and reactivity to such injuries as fracture, rickets, hyperparathyroidism, and so on. The same tumor attacking bone at these different sites should show a similar gradient, structurally and functionally. Homology, architecture, and the indicated differences in their structure and behavior indicate that osteogenic sarcoma, giant cell tumor and osteoid osteoma are genetically identical. Chondromas are derived from the epiphyseal plate. The neoplastic nature of diaphyseal aclasis is rejected.

THE TIME FACTOR IN THE IRRADIATION OF MALIGNANT TUMORS. Perry J. Melnick and (by invitation) Albert Bachem, Chicago, Ill.

*Abstract.* The purpose of this experiment was to study the histology of tumors irradiated by divided dose methods, as well as to compare the various X-ray treatment methods under controlled conditions. One hundred thirty-one transplantable rat tumors were treated by the massive, fractional, modified protracted-fractional and saturation methods. The results of greatest interest are the demonstration of two different types of degenerative changes in irradiated tumor cells. One of these is well known—the primary necrosis seen in sensitive cells consisting of a characteristic pyknosis and karyorrhexis of the nucleus. The other type of change is seen in refractory tumor cells irradiated by divided dose methods. It consists of the alteration of the nuclei of the cells so that they are transformed into abnormal forms which fail to survive, a kind of lethal mutation. The refractory cells in some of these tumors were transformed, under divided dose methods of irradiation, into practically pure cultures of hyperchromatic giant cells which degenerated in a specific manner by calcification of their nuclei.

### *Discussion*

(Dr. W. C. Hueper, Wilmington.) Several years ago I studied the mineral content and distribution of tumor tissue in incinerated sections prepared from fresh unfixed tissue. It was noticed at that time that tumor cells in the early stages of degeneration showed an increased content of calcium of the nuclei (central calcification). I wonder therefore whether the changes just reported can be considered as specific or whether they represent a phase of the degenerative process commonly found.

(Dr. Shields Warren, Boston.) In the material which Dr. Brues reported the day before yesterday with the use of an agent which injures the nuclei, colchicine, one gets a failure of mitosis to carry through an organized separation of the chromosomes, and frequently individual chromosomes form minute multiple nuclei within the cell which subsequently fuse and form nuclei bizarre in shape and in form, rather resembling the nuclei Dr. Melnick has shown. I should like to inquire whether in the early stages of these processes such scattered nuclei appear.

(Dr. Melnick.) I was able to follow various stages of calcification from the very earliest to the far advanced which you saw on the slide. It is my impression that this is probably a specific degenerative change, although I cannot prove it.

As far as fusion of nuclei is concerned, I saw multinucleated giant cells apparently arising from fusion of several tumor cells, especially in the tumors treated by the massive dose technic; apparently a surface tension change. In these mononuclear giant cells I saw no clear-cut evidence of fusion of multiple nuclei. They divide and we see enormous bizarre mitotic figures, which seem to be individual units.

THE RELATION OF CHRONIC MASTITIS TO CARCINOMA OF THE BREAST. Shields Warren and (by invitation) J. R. E. Morgan and John Fallon, Boston, Mass.

*Abstract.* To determine the possible precancerous character of breast lesions, we have studied the end results of 783 cases of so-called chronic mastitis and chronic cystic mastitis. Some cases were operated on in Boston and some in Toronto. Five hundred and forty-nine had only excisional biopsy done; the remainder had simple unilateral mastectomy. The follow-up period ranged from 5 to over 20 years, and averaged over 8 years.

Forty-seven cases had recurrence of non-neoplastic breast disease, either in the same, the opposite, or both breasts. Twenty-four cases of breast carcinoma developed, in 3 cases so soon that probably the biopsy failed to demonstrate an already existing carcinoma.

We have calculated the number of cases of breast carcinoma that would be expected in this group, based on the Massachusetts female population of 1930. Several methods of estimating the expectancy were used, and under 4 cases of breast carcinoma should develop in the group during the follow-up period. The actual number (21) is sufficiently greater than the expected to indicate breast disease as a predisposing factor to carcinoma.

As yet criteria are unsatisfactory for determining the lesions most likely to become carcinomatous. Unilateral mastectomy does not protect. Since less than 2.5 per cent of females in this group have developed carcinoma, careful checking for early clinical evidence of malignancy would seem more rational as a prophylactic measure than bilateral mastectomy.

THE RELATION OF MULTIPLE MENINGEAL TUMORS TO VON RECKLINGHAUSEN'S NEUROFIBROMATOSIS. Percival Bailey, Chicago, Ill.

*Abstract.* A study has been made of two girls, 15 years of age at the time of death, who suffered from multiple tumors of the nervous system. One girl had generalized subcutaneous and cutaneous manifestations of neurofibromatosis. She died following an attempt to remove a tumor from the cerebello-pontine angle. At autopsy there were neurofibromas on nearly all the roots of the spinal nerves, a neurofibroma of the twelfth nerve, a glioma of the optic chiasm, a glioma of the cerebellum and nodules of neuroglia cells in the cerebellar cortex; also all the nerves of the body were enlarged and showed the findings of the interstitial hypertrophic neuritis of Dejerine-Sottas. The other girl died following the attempt to remove a tumor from the cerebellar region. At autopsy were found multiple neurofibromas of the roots of the spinal nerves, of the cranial nerves, and interstitial hypertrophic neuritis of the nerve roots, also multiple meningeal tumors, both intracranial and intraspinal, and in addition the typical findings of tuberous sclerosis in the cerebral cortex. The essential relation of the leptomeninges to the other covering cells of the nervous system is again confirmed by these cases.

MULTIPLE TUMORS OF THE SYMPATHETIC NERVOUS SYSTEM WITH A REPORT OF A CASE ILLUSTRATING BOTH BENIGN AND MALIGNANT TYPES. H. R. Wahl and (by invitation) P. E. Craig, Kansas City, Kan.

*Abstract.* Tumors of the sympathetic nervous system are not common, and may be undifferentiated in structure and malignant in action, or may be be-

nign and well differentiated growths. The latter may be either a ganglioneuroma composed of ganglion cells and nerve fibers, or a paraganglioma, usually arising in the medulla of the adrenal glands and made up of chromaffin cells. Multiple tumors may occur but are very rare. The case reported is that of a young adult negro with a retroperitoneal neuroblastoma combined with two separate retroperitoneal ganglioneuromas, one of which showed extensive secondary hemorrhage and calcification and contained clusters of undifferentiated nerve cells or neurocytes.

### *Discussion*

(Dr. Louise H. Meeker, New York City.) At a recent autopsy on a female 75 years of age an unexpected finding within the duodenum was a mucosal polyp about the size of the thumb; the enlarged polypoid end contained a tumor beneath the mucosa which we have called a paraganglioma, and in that diagnosis Dr. Ewing has concurred.

(Dr. Frederic Parker, Jr., Boston.) I should like to ask what type of tumor metastasized to the vertebrae.

(Dr. Norbert Enzer, Milwaukee.) Were the adrenal glands involved?

(Dr. Wahl.) The adrenal glands were not involved.

The metastases to the vertebrae were apparently of the neuroblastoma type.

### EPITHELIAL METAPLASIA OF THE THYROID WITH SPECIAL REFERENCE TO THE HISTOGENESIS OF SQUAMOUS CELL CARCINOMA OF THE THYROID. R. H. Jaffe, Chicago, Ill.

*Abstract.* In 3 cases of sclerosis of the thyroid and near a metastatic abscess to the thyroid complicating a malignant endocarditis, metaplasia of the epithelium of the follicles to squamous epithelium was observed. This metaplasia resulted in the formation of islands of squamous epithelial cells which occasionally revealed epithelial fibrils. The islands were surrounded by argentaffine fibrils similar to the follicles. It is suggested that epithelial metaplasia may be the source of the rare squamous cell carcinomas of the thyroid, and it is therefore not necessary to trace this type of tumor to embryonic structures, such as the branchial clefts, thyroglossal duct or ultimobranchial bodies. In 1 of 3 cases of squamous cell carcinoma of the thyroid, epithelial metaplasia was found in follicles, in addition to the tumor.

### *Discussion*

(Dr. Stanley Reimann, Philadelphia.) If that is so for the thyroid gland, is it not also true for organs with occasional squamous epithelial lining, such as the gall-bladder, or the urinary tract, or other places where ordinarily there are tall cylindrical cells? In other words, the potentiality of undifferentiated cells—I call them “spare parts”—is at least two; they can turn into tall cells, or into squamous cells. The environment determines which of the two potencies is to be expressed. In Dr. Jaffe’s case an abscess determined squamous cells. If you feed an animal a diet deficient in vitamin A, the lining of the trachea turns into squamous epithelium. I am afraid Cohnheim’s theory is rapidly becoming of historical interest.

(Dr. Kornel Terplan, Buffalo.) I saw in very rare instances squamous cell



metaplasia in the thyroid. I recall 1 case following severe iodine treatment for Graves' disease in which the surgically removed thyroid showed in addition to typical changes of Graves' disease a very severe interstitial thyroiditis, islands of squamous cells and attempts at keratinization. There were also structures resembling the well known pseudotubercles, made up of so-called epithelioid cells and giant cells of unquestionably epithelial origin.

I should like to ask Dr. Jaffe whether retention or stagnation of colloid in these sclerosing conditions may induce the metaplastic change of the epithelium in the follicles of the thyroid.

(Dr. Shields Warren, Boston.) I should like to ask what the approximate frequency of these changes may be. They have been comparatively rare in my material, I should say less than ten instances among perhaps 12,000 thyroids, and I should also like to ask whether there is any one type of lesion with which they are more apt to occur. In a great many thyroids showing the so-called foreign body reaction to colloid, I have seen them very rarely. On the other hand, where there has been an inflammatory process, such as an abscess, as Dr. Jaffe described, I have the impression they are a bit more frequent.

(Dr. Jaffe.) As far as Dr. Reimann's discussion is concerned, metaplasia may occur in any epithelium wherever tissue breaks down and tissue regenerates and the proliferating cell meets with changed environmental conditions. Not so long ago a case of squamous cell carcinoma of the head of the pancreas came under my observation in which the tumor had developed from the metaplastic epithelium of the pancreatic duct.

Concerning the retention of the colloid, I do not think it is related to this condition, because the follicles first lose their colloid content, then shrink, while some of the epithelial cells degenerate and others proliferate and gradually assume the appearance of squamous cells.

As far as the incidence is concerned I cannot give you an exact figure, but the cases I have presented were found among several thousands of thyroids examined microscopically.

Dr. Warren is right — metaplasia of the follicular epithelium occurs chiefly in inflammation.

The tubercle-like lesions are entirely different. I described them a number of years ago. They are not composed of squamous epithelial cells but of epithelioid cells which are derived from the follicular epithelium.

HISTOPATHOLOGY OF MIXED TUMORS OF THE SALIVARY GLANDS. John W. Budd, Los Angeles, Calif.

*Abstract.* This study is concerned with the various histological patterns observed in a series of salivary gland tumors. It is believed that the entire group forms a continuity with the following factors responsible for the unusual structures: (a) degree and direction of differentiation of the epithelioblast; (b) chemical nature of the secretion; and (c) direction of the secretion.

### *Discussion*

(Dr. W. C. MacCarty, Rochester, Minn.) Having been something of a technical purist all my life, my esthetic as well as scientific sense has often been offended in these meetings. I have been attending them for nearly 30

years, and the microscopic material which I have seen thrown on the screen has been terrible. Therefore I want now to compliment this young man (I do not know him), on showing the most beautiful histological and cytological pictures I have seen at these meetings. I hope he will keep it up and not fall back into some of the old-fashioned methods of histological and cytological studies.

(Dr. W. S. Hastings, Philadelphia.) I am much interested in Dr. Budd's belief, if I understand him correctly, that this material in the stroma is a secretion from the epithelial cells. In certain instances, at least, I have been strongly inclined to the same view. I should like to ask him, however, how he accounts for the frequent occurrence of cartilage in these tumors.

(Dr. Budd.) I would refer Dr. Hastings to an article by Masson, who can explain the cartilage formation in these tumors far better than I can, but it is merely an accentuation of the process which I attempted to represent.

HISTOLOGICAL STUDIES OF TUMOR REACTIVITY TO BACTERIAL FILTRATES. Isadore E. Gerber and (by invitation) Alice I. Bernheim, New York City.

*Abstract.* Mice bearing Sarcoma 180 were given a single intravenous injection of 20 to 100 reacting units of meningococcus agar-washings filtrate. The tumors were examined grossly and microscopically with a view to understanding the mechanism underlying the tumor response. The filtrate employed was capable of eliciting the Shwartzman phenomenon in rabbits. One hundred and thirty mice were treated from 3 to 12 days after tumor inoculation, the majority at 10 days, and observed from 4 hours to 10 days after treatment. This series includes only animals surviving the injection of the filtrate.

Six day or older tumors all responded in varying degrees dependent upon the amount of filtrate used. There were severe degenerative changes, striking vascular engorgement, edema and necrosis of the tumor cells followed by either complete regression or regrowth of the tumor from surviving cells. Hemorrhage was seen in the tumor only in the early period (4 hours) following filtrate administration. Lesions of the vessel walls or thrombi were not noted. The effect of the filtrate appeared to be directly on the tumor cells.

Many experimental observations, including our own, suggest that the reaction of the tumor described is analogous to, and is elicited by, the bacterial active principles of the Shwartzman phenomenon. Since no preparatory local injection is required, and since a single intravenous injection is sufficient to call forth the reaction, it appears that the growing neoplastic tissue spontaneously acquires a state of vulnerability.

### *Discussion*

(Dr. M. J. Shear, Boston.) In a good many respects the results obtained in our laboratory are in agreement with those Dr. Gerber has reported. I may add that primary sarcomas produced in mice by hydrocarbons are also susceptible to the action of such filtrates. Our more recent results are in agreement with what Dr. Gerber has just said about the similarities between the hemorrhage in mouse tumors and the Shwartzman phenomenon. Filtrates obtained from *B. coli* were fractionated, and the agent that produced hemorrhage in mouse tumors was separated from accompanying impurities and a highly potent fraction obtained. At this point the material that produced

hemorrhage in mouse tumors in doses of a fraction of a millionth of a gram was then injected into rabbits; it was found that this same material produced hemorrhages in the skin of rabbits, *i.e.* the Schwartzman phenomenon, in doses of the same order of magnitude. I should like to ask Dr. Gerber whether it is true that mice are not susceptible to the Schwartzman phenomenon, as are rabbits, and if that is so, whether he has any explanation as to why mouse tumors should be susceptible.

(Dr. Gerber.) I can answer only the first part of Dr. Shear's question. Mice do not show the Schwartzman phenomenon, as originally defined by Schwartzman. Why the mouse tumor should show it is impossible to answer at the present time.

**BOECK'S DISEASE. POSTMORTEM FINDINGS IN A CASE WITH VISCERAL LESIONS.**  
Max Pinner, Ithaca, N. Y.

*Abstract.* The autopsy findings in a case of Boeck's sarcoid are presented. The characteristic granulomas, consisting of miliary nodules of epithelioid cells without, or with a paucity of other tissue changes, were present in the skin, many lymph nodes, lungs, pericardium, kidneys and spleen. In this particular case the potential developments of granuloma can be demonstrated in a rather convincing manner. They are (1) a singularly coarse hyalinized fibrosis, and (2) caseation—that is, transformation into banal tuberculosis which, however, is distinguished by practically complete absence of exudative features. Hyaline fibrotic nodules, evidently late stages of epithelioid granuloma, were found in all organs mentioned except the skin, and in the bone marrow of phalanges and sternum, causing in the former the characteristic roentgenological appearance of osteitis tuberculosa cystoides multiplex (Jüngling). Caseated nodules were found in all organs mentioned and in the myocardium, but not in the skin. In the lungs the process led to a diffuse, hyaline interstitial fibrosis. A study of this case, supported by considerable evidence from the literature, leaves little doubt that Boeck's sarcoid is a particular form of tuberculosis.

*Discussion*

(Dr. Ralph D. Lillie, Washington, D. C.) Some of the cases of Boeck's disease in which we have seen the cutaneous manifestations were definite cases of maculoanesthetic or nerve leprosy. In hearing Dr. Pinner's presentation I wondered whether the caseation, and the appearance of acid-fast bacilli, and the cavitation of the lungs were a part of the Boeck's disease, or the supervening of an active tuberculosis on a possibly leprous lesion, a thing that is a very common finding in leprosy when it is institutionalized.

(Dr. Henry C. Sweany, Chicago.) I have been very much interested in this presentation because it is near to my heart. Many years ago we had an experience with certain types of tubercle bacilli that were not what we would call typical, and I believe that pathologists in general should look to the variations in the tubercle bacillus for variations in pathology more than they usually do. Pathologists have attempted to explain much variation in pathology on the basis of changes in the host and constitution, dosage, allergy, and so on, but I do not believe there has been enough attention paid to these rare occasional offshoots, which we may call variations at certain times and, if we

choose, mutations at others. Smithburn, for example, has driven a very fine wedge into this problem by showing that we can vary almost any strain of tubercle bacillus by varying the hydrogen ion concentration, and these variations produce variations in pathology, according to the variation in the bacillus. I believe that many of these variants do produce pathological changes that parallel the variation. I want to ask Dr. Pinner, however, if he cultured these lesions, if he obtained growths from the culture or animal inoculation and, if so, what was the characteristic of the growth?

(Dr. Frederic Parker, Jr., Boston.) As I understood Dr. Pinner, he said there was caseation present. In making the diagnosis of the cases of Boeck's disease I have seen, we thought the presence of caseation automatically ruled out the diagnosis of sarcoid. I should like to ask Dr. Pinner concerning this point.

(Dr. Pinner.) I think I can say very definitely that this case is not leprosy. The skin lesions were quite slight and there was no clinical evidence even to suggest leprosy. I am familiar with the cystic bone lesions in leprosy that have a similar appearance to the ones shown in this case, but this is the only similarity between leprosy and the case presented.

As far as the bacteriology is concerned, I was afraid that would come up. We did make cultures and we did not get any growth from the lymph nodes. We did not make animal inoculations because our laboratory at the time was in a very embryonic state and we were unable to do it for technical reasons.

As far as the question of caseation is concerned, I am perfectly willing to agree that caseation should exclude the diagnosis of Boeck's disease. The skin lesions and the lymph node lesions which I showed at first were characteristic of Boeck's disease and did not show any caseation. As a matter of fact, the first two slides, the one from the skin and the first lymph node lesion, were biopsy material on this patient. On that basis we felt justified in making a diagnosis of Boeck's disease. When caseation supervenes, I think, it is entirely a question of taste whether one wishes to say that now Boeck's disease has become tuberculosis, or whether one chooses to say that caseation is a potential development of Boeck's disease. In my opinion, not based only on this case but on an analysis of the literature as well, the entire terminology of Boeck's disease, or benign miliary lupoid, is entirely unnecessary. All we have to say is that so-called Boeck's disease is a particular type or phase of tuberculosis, which, similar to any other form of tuberculosis, has the two potential developments, fibrosis and caseation, as demonstrated in this case.

Going back to Dr. Sweany's discussion, in spite of the fact that we were not able to isolate the organism, I think the evidence is definitely in favor of assuming that the particular type of lesion characterized as Boeck's disease is not due to any variation in the bacillus, but to an intrinsic factor, because a very large percentage of patients with these lesions are anergic to tuberculin. So was this patient until 4 weeks before his death. Up to that time his sputum on many occasions was negative. Four weeks before death he showed tubercle bacilli in the sputum and tuberculin allergy. Tuberculin anergy and absence of caseation seem to be closely associated; which is the cause and which the effect we do not know, but there is undoubtedly a close relation between caseation and tuberculin allergy.

THE EFFECTS OF COAL SMOKE OF KNOWN COMPOSITION ON RABBIT LUNGS.  
Lucy Schnurer (by invitation) and Samuel R. Haythorn, Pittsburgh, Pa.

*Abstract.* Bituminous coal was burned continuously in an egg stove over a period of 80 days and about 10 per cent of the flue smoke diverted, mixed with oxygen and passed through air-tight chambers containing animals. Dust counts and analyses for gas contents were made daily. The smoke particles were 75 per cent carbon, and the ash contained 1.1 per cent silica particles by count. Some of the animals were killed at the end of the exposure and the rest were returned to animal house conditions to be examined at varying periods. The last set was examined over a year after the exposure had ceased. Immediately after exposure the lungs contained great quantities of carbon phagocytes and much free carbon. Later the phagocytes began to be collected in clumps in the alveoli and in various sets of lymphatics. The final animals showed many nodular collections surrounded by areas of pneumonitis and interspersed with collagen fibrils. The pigmented phagocytes in the lymph nodes first examined were diffuse. In the later nodes they were gathered in large confluent masses. The lung changes resembled those of persons living in smoky atmospheres rather than those associated with occupational diseases. The results indicated that carbon in sufficient quantities is capable of producing fibrous changes. A way was found to prepare the lungs of rabbits for further studies involving the relation of smoke and pulmonary infections.

*Discussion*

(Dr. Arthur J. Vorwald, Saranac Lake.) What was the method of making the dust counts? Was it by light or by dark field?

(Dr. Haythorn.) I did not make the dust counts. They were made by light field at 970 diameters. Another thing I neglected to say was that we did incinerations of all these sections and could find no crystalline substance in any of the incinerated residues.

(Dr. Vorwald.) Is it possible that substances present in the smoke, other than the dust *per se*, might have been responsible for the pulmonary fibrosis observed in the experimental animals? I am referring particularly to the gases that were liberated and subsequently inhaled by this method of experiment.

(Dr. Haythorn.) The only chemical analysis I have here showed no other substances except those that were given, namely oxygen, carbon monoxide, carbon dioxide, hydrogen sulphide, and sulphur dioxide, and the dust particles. We do not know what was in the ash fraction. There were several amorphous things there not identified, and it was not impossible that there were some other substances. However, this kind of smoke is the sort of thing we go around breathing all the time and we find that it does not increase the incidence of tuberculosis. The lung picture is the same that we have found associated with much organizing pneumonia in our district.

## READ BY TITLE

- THE QUARTZ CONTENT OF SILICOTIC LUNGS DETERMINED BY THE X-RAY DIFFRACTION METHOD. Dudley A. Irwin, Toronto, Canada.
- THE METEOROLOGICAL INFLUENCE ON THE OCCURRENCE OF HEMORRHAGE AND PERFORATION IN PEPTIC ULCER. George Milles (by invitation), Chicago, Ill.
- ETIOLOGICAL FACTORS OF APPENDICITIS MORTALITY. Frederick W. Mulsow, Cedar Rapids, Ia.
- THE COMPARATIVE THICKNESS OF NORMAL, SULFHYDRAL-TREATED, AND DISULFOXIDE-TREATED MOUSE SKINS. Stanley P. Reimann, Philadelphia, Pa.
- HISTOLOGICAL STUDIES OF A MUSCLE HEMANGIOMA. George Rukstinat, Chicago, Ill.
- MORPHOLOGICAL VARIATIONS OF TUMOR CELLS. Otto Saphir, Chicago, Ill.
- COMPLETE OCCLUSION OF THE ENTIRE VENA CAVA BELOW THE HEPATIC VEINS. A. L. Sparks (by invitation), Cleveland, Ohio, and Herbert Fox, Philadelphia, Pa.
- THE PATHOLOGY OF OMENTAL CYSTS. F. W. Wigglesworth, Montreal, Canada.
- VASCULARITY OF THE BLOOD VESSEL WALL. ADAPTIVE CHANGES. Milton C. Winternitz and (by invitation) Robert M. Thomas, New Haven, Conn.



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## ARTERIOLAR SCLEROSIS IN HYPERTENSIVE AND NON- HYPERTENSIVE INDIVIDUALS \*

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### INTRODUCTION

The association of cardiac enlargement and renal contraction has been recognized since Bright's classical case reports in 1836. Shortly thereafter Johnson added diffuse disease of the smallest arteries to the pathological anatomy of Bright's disease and in 1873 proposed the theory that renal disease was primary with subsequent diffuse thickening of the walls of the smallest arteries leading to increased peripheral resistance, elevated blood pressure and cardiac hypertrophy.

Subsequent observations added complexity rather than simplification to the problem. Gull and Sutton reported widespread small vessel disease called by them "arterio-capillary fibrosis," and observed that "these changes are, or may be, independent of renal disease, and that the renal change in chronic Bright's disease with contracted kidneys, when present, is but a part of a general morbid condition." They concluded that the diffuse vascular disease was a primary pathological change responsible for

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increased resistance to blood flow. Confirming this general hypothesis were the early clinical observations on blood pressure by Mahomed to the effect that high blood pressure precedes the clinical signs of renal damage.

There were then three divergent views as to the pathogenesis of chronic hypertension: (1) that the renal disease was primary; (2) that the renal disease was but a part of a diffuse primary vascular disease; and (3) that the hypertension itself was primary, the renal and the vascular changes being secondary to it.

The recognition by Jores of two types of contracted kidneys, inflammatory and arteriosclerotic, provided the anatomical basis for differentiating nephritic from essential hypertension. The latter, according to Fishberg's definition which is representative of the majority opinion, is chronic hypertension which neither on clinical nor anatomical grounds can have been caused by preceding inflammatory or obstructive renal disease. It has become generally accepted that the primary change in nephritic hypertension is the renal injury, but the relation of the contracted kidney of nephrosclerosis to hypertension has remained a controversial subject. It is generally believed that the renal vascular disease leads to renal atrophy and contraction but there is a divergence of opinion about the relation as to the cause and effect between the hypertension and the renal vascular disease. Certainly, essential hypertension commonly precedes any clinical evidence of renal insufficiency. Whether or not chronic hypertension precedes the vascular disease that so frequently leads to renal atrophy is the controversial point.

This divergence of opinion as to the relation between essential hypertension and vascular disease may be illustrated by citing the opinions of Volhard and Fahr. Volhard recognized two principal types of hypertension, "pale" and "red." "Pale" hypertension is secondary to renal insufficiency. The renal insufficiency may be due to primary renal disease (glomerulonephritis, and so on) or to the renal vascular damage caused by "red" hypertension, and the "pale" hypertension is the immediate result of the action of pressor substances causing spasm of arterioles throughout the body. "Red" hypertension is primarily a functional derangement to which certain persons are constitutionally predisposed and in which the general distensibility of small arteries is impaired and

blood pressure elevated entirely without primary organic arteriolar contraction. In regard to the "red" or "essential" hypertension his views are based on "the assumption of an early senescence of the whole arterial system, a fatigue of its different structural units, microscopically visible in diminution of internal muscular layer, defects in the elastic layer and over-stretching, all processes commencing while a normal pressure still exists and followed by an increased tonus of the arterioles via vaso-vascularis reflex." This places the cause of essential hypertension outside the kidney except in those instances where the hypertension itself causes renal damage and thus establishes a vicious circle. Fahr, on the other hand, was of the opinion that hypertension is a secondary phenomenon in relation both to inflammatory and to arteriosclerotic renal disease. The primary change is renal and the mechanism by which blood pressure is elevated is, according to him, "compensatory." Bell and Clawson have attempted to harmonize these contradictory views by admitting the absence of any real evidence as to which is antecedent, hypertension or renal vascular disease, but opined that "it is, however, probable that when severe arteriolar sclerosis of the kidney has developed, the circulatory obstruction tends to raise the blood pressure to higher levels."

Critical commentators, such as Hewlett and Boyd, have expressed the belief that the primary change in essential hypertension is functional in the form of arteriolar spasm with resulting increase in peripheral resistance and a later development of permanent structural changes in the form of arteriolar sclerosis which fixes the hypertension by organic narrowing of vessels. Christian and O'Hare felt that the present evidence as to the cause and effect relation of chronic hypertension and arteriolar sclerosis is inadequate to support any definite conclusion as to sequence.

Excluding such possible causes of hypertension as nephritis, urinary obstruction, obesity, hyperthyroidism, pituitary tumor, lead poisoning, adrenal tumor, aortic insufficiency, coarctation of the aorta and arteriovenous aneurysm, there remain the following general conceptions as to the cause and effect relation of arteriolar sclerosis and chronic hypertension:

1. That chronic hypertension is the result of primary renal arteriolar sclerosis, either because a generalized reflex spasm of peripheral vessels is initiated in the ischemic kidneys, or because

of the retention or elaboration of pressor substances incident to a reduced blood flow through the kidney.

2. That chronic hypertension is the result of increased resistance to blood flow caused by widespread primary arteriolar sclerosis with resulting narrowing of vessel lumens.

3. That chronic hypertension is due primarily to arteriolar spasm and that the arteriolar degenerative changes in the kidney and elsewhere are secondary to the increased intravascular pressure and may or may not serve to increase the severity of the hypertension because of organic lumen narrowing.

There is an almost equal lack of agreement as to the character and distribution of vascular lesions in association with essential hypertension. Johnson and Ewald described medial hypertrophy as being a predominant arteriolar change and observed it as occurring generally throughout the body. Gull and Sutton described an equally wide distribution of the arteriolar lesions but believed them to represent degeneration rather than hypertrophy. Jores reported widespread arteriolar intimal hyalinization but failed to find vascular changes in the skeletal muscles. Evans observed medial hypertrophy as well as intimal proliferation and hyalinization in many organs of hypertensive individuals but together with Fishberg and Bell and Clawson failed to discover arteriolar disease in the skeletal muscle. The interest that has been manifested in the occurrence or non-occurrence of arteriolar disease in the skeletal muscle in essential hypertension has been stimulated by the fact that the skeletal muscles constitute so large a part of the peripheral vascular bed. Kernohan, Anderson and Keith reported that the arterioles in skeletal muscles of persons with essential hypertension very frequently showed hypertrophy of the media, proliferation of the intima and reduction of lumen caliber. Scott, Seecof and Hill reported a high incidence of arteriolar lesions in the skeletal muscle of hypertensive patients and Andrus observed more severe fibrosis of the media of arterioles from the skeletal muscles of hypertensive than of non-hypertensive individuals but did not believe that there was wall thickening or reduction of lumen caliber.

The determination of the relation that arteriolar disease bears to chronic hypertension is obviously of more than academic interest especially with the advent of the surgical treatment of the disease.

If permanent organic changes are primary, less can be expected of operations designed to relieve vascular spasm than if the vascular spasm were primary and the organic changes secondary. In the elucidation of the problem it was felt that a clear understanding of the pathological anatomy of hypertension would be of value. Many careful and comprehensive investigations of the pathological anatomy of hypertension have been made and it appeared that the only hope for further morphological or statistical investigations would be the employment of different methods. So far as could be determined no purely objective and yet comprehensive study of the pathological histology, distribution and relative severity of arteriolar lesions in the various organs and tissues of a large number of individuals has been heretofore reported. The correlation of these objective findings with the clinical and anatomical data assembled later was undertaken in the present investigation.

Prior to this objective study of arteriolar disease a general survey was made of the clinical and pathological characteristics of cases of essential hypertension based on the records of the departments of medicine and pathology of Western Reserve University and The University Hospitals.

#### GENERAL CHARACTERISTICS OF THE POPULATION OF A REPRESENTATIVE CHRONIC HYPERTENSIVE GROUP

The clinical and autopsy records of 200 consecutive cases of chronic hypertension were studied to determine the age, race and sex characteristics of the disease. These were cases in which there was no significant degree of inflammatory heart disease. They were known to have had prolonged elevation of systolic blood pressure over 150 and diastolic pressure over 100 mm. of mercury, and to have had heart weights in excess of 400 gm. in males and 350 gm. in females. In the presence of obliterative coronary arterial disease heart weights in excess of 500 gm. were required for acceptance. No case was included in this series unless death was due to uremia, cardiac decompensation or cerebral hemorrhage.

The group was comprised of:

116 males	with a mean age of	52 years			
84 females	" " " "	50 "			
60 blacks	" " " "	47 "	P.E.m.	0.9	
140 whites	" " " "	55 "	P.E.m.	0.5	

In this series the mean age of females was 2 years lower than the age of males at the time of death. This was not a statistically significant difference. The mean age of blacks was 8 years lower than that of the whites at the time of death which was 7.5 times greater than the P.E. of the difference.

A comparison of the race and sex population of the hypertensive group to the race and sex population of 1177 consecutive autopsies of all kinds performed on individuals over 30 years of age between the years 1930 to 1935 in the same laboratory is shown below.

	Whites	Blacks	Males	Females
	%	%	%	%
Cases of chronic hypertension .....	70	30	58	42
General autopsy series .....	80	20	60	40

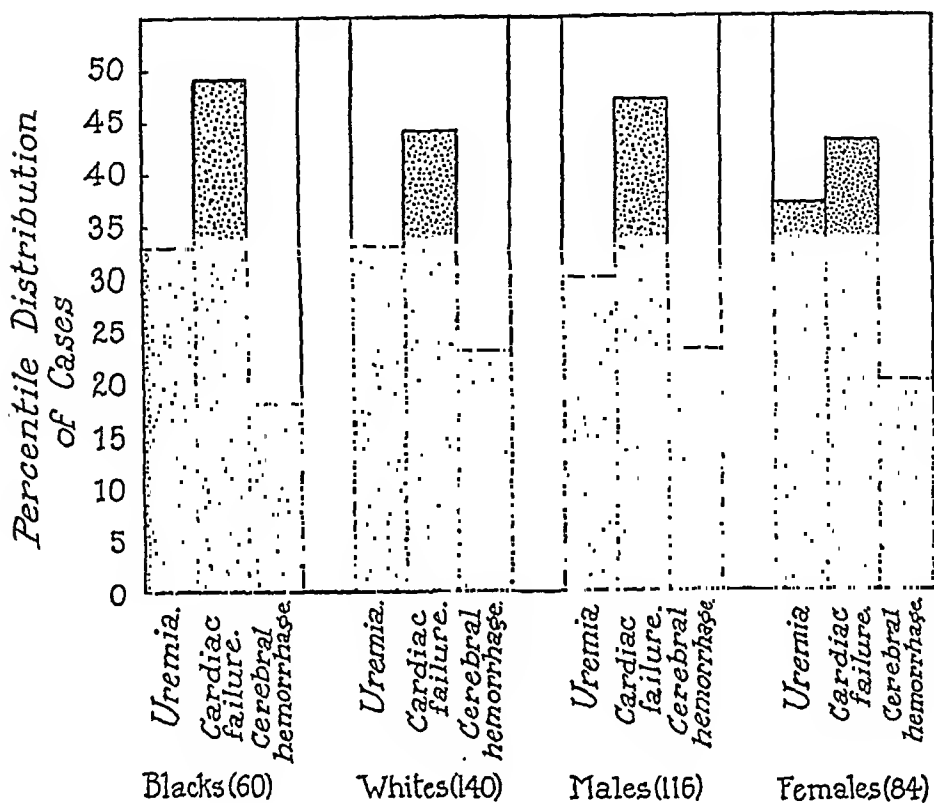
The only significant difference in the population of the chronic hypertensive group as compared to that of the general autopsy series is a racial one. Blacks were encountered with greater relative frequency in the hypertensive group than would be expected from the racial constitution of the general autopsy series. There are 50 per cent more blacks in the hypertensive group than the incidence of the blacks in the general autopsy population would indicate. In contrast there are 13 per cent fewer whites in the hypertensive group than would be expected if whites and blacks had the same degree of mortality from conditions related to chronic hypertension.

Two conclusions appear to be justified as far as this group of 200 cases is concerned: one is that the blacks with chronic hypertension died at a younger age than did whites; and the other is that the percentage of blacks in the hypertensive group was higher than the percentage of whites if the racial constitution of the entire autopsy population was considered.

An investigation was next made of the relation of race and sex to the mode of death in cases of essential hypertension. In the entire group it was found that there were 68 cases in which death was due to uremia, 89 to cardiac decompensation and 43 to cerebral hemorrhage. In Text-Fig. 1 it may be seen that in the different race and sex groups there were no very striking differences in the proportion of each group dead of uremia, cardiac decompensation and cerebral hemorrhage.

The greater susceptibility of blacks over whites to death from

hypertension or its complications was not reflected in the manner in which blacks with hypertension died. About the same percentile proportions of death from uremia, cardiac decompensation and cerebral hemorrhage were seen in blacks, whites, males and females.

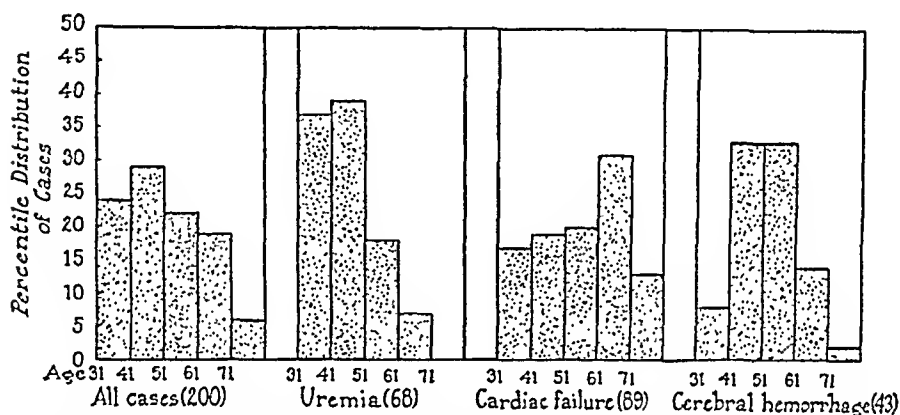


Text Fig. 1. Relation of race and sex to mode of death in 200 consecutive cases of chronic hypertension.

The correlation of age and mode of death showed striking differences in the age at death of the various types of chronic hypertension. In Text-Fig. 2 it may be seen that the highest incidence of death from uremia was in the 4th and 5th decades. Seventy-five per cent of all the individuals dead from uremia died under 51 years of age. The age incidence was quite different in the groups dead of heart failure and cerebral hemorrhage. In these, death occurred after the age of 51 in 64 per cent and 49 per cent respectively.

Of some significance is the high incidence of obliterative coro-

nary arterial disease in the group of chronic hypertensives dead of heart failure. Forty per cent of all those dead of heart failure were found to have either remote coronary thrombosis or obliterative coronary sclerosis. The incidence of severe coronary arterial disease in the chronic hypertensives dead of cardiac decompensation began at 27 per cent in the 4th decade and rose to 60 per cent in the 7th.



Text Fig. 2. Age at death of 200 consecutive cases of chronic hypertension in which death was due to uremia, cardiac failure or cerebral hemorrhage.

### ARTERIOULAR DISEASE IN HYPERTENSIVE AND NON-HYPERTENSIVE INDIVIDUALS

*Selection of Cases:* The material on which the major part of this study was made was comprised of 200 cases: 100 had no history of hypertension and the other 100 were known to have had chronic hypertension. Obviously, the possibility of hypertension having been present at some time in some members of the control group could not be positively excluded. The acceptance of a case as non-hypertensive depended on the fulfillment of two criteria. One was that there must have been repeated blood pressure determinations, none of which exceeded 140/90 and the heart weights in males were required to be less than 400 and the females less than 300 gm. As a further means of excluding cardiac hypertrophy, all hearts were examined microscopically and any showing histological evidence of hypertrophy were excluded. All cases selected for the chronic hypertensive group had repeated blood pressure determinations higher than 160/90 or 150/100. No male

was included in this group if the heart weighed less than 450 gm. and no female if the heart weighed less than 350 gm. Cases of inflammatory heart disease were excluded and if severe coronary arteriosclerosis were present, a heart weight in excess of 500 gm. was required for acceptance. The frequent occurrence of cardiac hypertrophy leading to heart weights up to 500 gm. in non-hypertensive individuals as the result of focal myocardial ischemia and dilatation in severe coronary disease has been described by Moritz and Beck. These cases were selected on the basis of the above outlined criteria and with no knowledge as to the presence or absence of renal or vascular disease. The selection was made in such a manner that the population of the two groups was similar in regard to age, sex and color. Approximately one-third of each group was comprised of individuals between 31 and 45 years of age, one-third between 46 and 60, and one-third 61 years of age or over.

*Size of Vessels Studied:* No satisfactory definition of an arteriole was found, other than that the smallest arteries are arterioles. A variety of criteria have been proposed to distinguish between small arteries and arterioles (Maximow, Cowdry, Benninghoff). These include the number of layers of smooth muscle in the media, the existence of an internal elastic lamella and the external cross sectional diameter of the vessel. We have not found any of these distinctions useful and propose to use the terms "arteriole" and "small artery" interchangeably. This investigation, however, was directed at arteries that had an external diameter of 100  $\mu$  or less.

*Methods of Investigation:* Various staining methods were found useful in a study of the exact anatomical character of certain arteriolar lesions. In addition to hematoxylin-eosin, the most important methods used were Weigert's for elastic tissue and van Gieson's for connective tissue. In studying a particular type of lesion, sections were cut in uninterrupted series and were stained in rotation with hematoxylin-eosin, Weigert's, van Gieson's, the Wilder method for reticulum, and the Mallory-Heidenhain azan-carmine method for connective tissue. In vessels chosen to illustrate certain types of vascular change, the hematoxylin-eosin stained preparation of the vessel was photographed and then the artist made a colored drawing using the photograph for structural



orientation and the slide for color detail. The same section was then decolorized and restained by the van Gieson method. The original drawing served for structural orientation and a second drawing was made again using the slide for color detail. The section was decolorized and restained for elastic fibrils and this time the drawing of the elastic fibrils was either superimposed on the drawing made from the van Gieson preparation or a third drawing of the same vessel was made showing the distribution of the elastic tissue with a neutral counterstain of the rest of the vessel.

Another useful method for the investigation of the pathological anatomy of arteriolar lesions was by means of uninterrupted series of sections. Nine uninterrupted series varying between 100 and 400 in each were prepared from various tissues to study the longitudinal extent and distribution of different types of arteriolar lesions.

*Recording of Data:* All of the sections of a given organ or tissue in each of the 200 cases were examined objectively with no knowledge as to the presence or absence of hypertension and a record was made as to the kind and severity of the arteriolar disease present. The severity of the arteriolar changes was recorded as mild, moderate or severe by the symbols +, ++ and +++. If the arteriolar disease was focal in a given tissue, a note was made as to the kind of lesion and the fact that it was focal, but such disease was ignored in the general classification of the severity of arteriolar sclerosis in a given tissue. Every organ finally classified as being the seat of mild, moderate or severe arteriolar sclerosis was characterized by the presence of diffuse vascular disease. In many cases of mild, and in some instances of moderate or severe sclerosis, all of the arterioles were not affected, but invariably enough were changed to constitute a general rather than a local process within that organ or tissue.

These degrees of change were not comparable to one another in different tissues. Severe arteriolar sclerosis in a skeletal muscle was not of the same grade of severity or even the same kind of vascular disease as was present in severe arteriolar sclerosis in the spleen. Mild, moderate or severe, refer to the three grades of severity in the particular tissue under consideration.

## PATHOLOGICAL HISTOLOGY OF CHRONIC ARTERIOLOSCLEROSIS

*Intimal Hyalinization:* This was the most common form of chronic arteriolar disease observed and consisted of a subendothelial accumulation of homogeneous, acidophilic material which appeared to represent an infiltration or expansion of the ground substance between smooth muscle of the media and the endothelium. Vacuolar degeneration, either fatty or hydropic, was common and although there was considerable variation in staining reaction of the hyalin, it usually stained deep blue with the Mallory-Heidenhain azan-carmin and the Mallory connective tissue methods, yellow with the van Gieson, and green with the Masson trichrome light green stain. The disposition of hyalin varied, being sometimes in the form of circumscribed subendothelial plaques (Fig. 1), sometimes a subendothelial collar of uniform thickness, and sometimes annular but of irregular thickness, so as to displace the lumen to an eccentric position (Fig. 2). The longitudinal distribution of the hyalin along a vessel was also variable with normal segments interspersed between diseased segments. Not all arterioles with intimal hyalinization had narrowed lumens and some actually appeared to be dilated (Fig. 3). This apparent dilatation was thought to be due to an increased resistance to post-mortem contraction imparted to the vessels because of the hyalin, this view being supported by the frequent absence of undulation of the internal elastic lamella of such vessels.

The relation of the internal elastic lamella to the hyalin varied considerably. Although the principal mass accretion of hyalin was commonly inside of the internal elastic lamella (Figs. 1 and 3), it appeared that the hyalin actually enveloped the elastic lamella which in many vessels lay approximately in the center of the hyaline mass (Fig. 2). In such circumstances, elastic degeneration was invariable and was represented by swelling, disruption and dispersion of fibers with eventual complete disappearance.

*Medial Hypertrophy and Degeneration:* Two types of chronic medial change in the arterioles were observed. One was manifested by increased medial thickness due to an increase in the number or size of smooth muscle cells (Fig. 5), and the other was represented by a relative increase in the amount of intercellular collagen throughout the media (Fig. 6). Although these changes

did occur independently of one another, yet they occurred together (Fig. 6) with such regularity that in the grading of arteriolar lesions according to severity the two changes were treated as though they were part of the same process.

Variations in the degree of postmortem contraction of vessels seen in different individuals or in different vessels of the same individual frequently made it difficult to identify medial hypertrophy. When true hypertrophy of the media had occurred (Fig. 5) the increased thickness of the media was not associated with the same degree of undulation of the internal elastic lamella as was present in normal vessels (Fig. 4), which appeared to have thick walls because of excessive postmortem contraction.

In normal arterioles the media was seen to have three components — smooth muscle, intercellular collagen and occasional irregularly disposed elastic fibers. With the van Gieson method the collagen stained very faintly or not at all in normal arterioles (Fig. 4), but in arterioles the seat of medial degeneration, the increase in collagen was readily recognized (Fig. 6). The increase in collagen was not associated with penetration of the media by fibrocytes, was more pronounced in the inner than in the outer half of the media, and was in some instances associated with atrophy rather than hypertrophy of smooth muscle cells. Medial degeneration showed marked segmental variation, as was the case also in intimal hyalinization. In some segments the medial degeneration was associated with severe secondary degenerative changes so as to cause stenosis of the lumen (Figs. 7 and 8). As a rule this change occurred independently of any intimal disease.

*Endothelial Hyperplasia:* Endothelial hyperplasia regularly resulting in reduction in lumen caliber was seen most frequently in vessels over 50  $\mu$  in diameter, occasionally in arterioles down to 30  $\mu$  in diameter, but rarely under this. As a rule the hyperplastic endothelium of the larger arterioles gave way gradually to intimal hyalinization in the smaller arterioles. It was not possible to recognize endothelial proliferation with certainty when all of the endothelium was represented by lining cells. In such circumstances postmortem contraction of the vessels together with tangential section could give an illusion of endothelial hyperplasia. The piling up of endothelial cells layer on layer did, however, present a clear picture of endothelial hyperplasia (Figs. 9 and 10).

Such hyperplasia was in rare instances superimposed on a relatively unaltered internal elastic lamella (Fig. 9). More commonly, however, there was formation of new elastic fibers between the hyperplastic endothelial cells. In some vessels this new elastic tissue formed an irregular intercellular mesh, which in others was seen to be organized to form more or less concentric lamellas, the lamellas being separated from one another by newly formed endothelial cells (Fig. 10). As in the case of the media, there was no recognizable penetration of the intima by fibrocytes although the proliferated endothelial cells tended to become spindle shaped so as to resemble fibrous connective cells closely. As in the case of intimal hyalinization, the elastic fibers, both new and old, showed a pronounced tendency to degeneration, as manifested by swelling, disruption and dispersion of fibrils. This degeneration of elastic fibers was especially pronounced when the proliferated endothelium underwent hyalinization, which was a common secondary degenerative change in vessels affected in this manner (Fig. 10). A wide variety of acute secondary degenerative changes was seen and will be described later.

#### PATHOLOGICAL CHANGES IN ARTERIOLES OF NON-HYPERTENSIVE INDIVIDUALS

As already indicated, this study was directed at chronic rather than acute arteriolar changes. Acute primary degenerative and inflammatory arterial and arteriolar disease have been discussed recently by Karsner and were not studied in this investigation.

The incidence and relative severity of arteriolar sclerosis in the various organs and tissues in non-hypertensives is summarized in Table I. The table does not include a record of the vascular changes in the female internal genitalia where cyclic hyperplasia and involution make their occurrence exceedingly common, or changes in various tissues not examined with sufficient frequency to make statistical comparisons profitable. The heart and lungs were not included in the table because of the rarity of arteriolar changes in those situations in this series of cases. Pulmonary (Parker and Weiss) and myocardial (Karsner and Bayless) arteriolar sclerosis is seen most commonly in cases of rheumatic carditis and since no individuals with cardiac enlargement were

TABLE I

*The Relation of Age, Sex and Race to the Incidence and Severity of Arteriol Sclerosis in Non-Hypertensive Individuals*

Age Groups	Spleen			Pancreas			Adrenal			Gastro-Intestinal			Brain *			Skeletal muscle			Liver			Kidney		
	Mild or moderate	Severe	Total	Mild or moderate	Severe	Total	Mild or moderate	Severe	Total	Mild or moderate	Severe	Total	Mild or moderate	Severe	Total	Mild or moderate	Severe	Total	Mild or moderate	Severe	Total	Mild or moderate	Severe	Total
31-45 yrs.	% 56	% 17	% 73	% 38	% 0	% 38	% 25	% 0	% 25	% 12	% 0	% 12	% ..	% ..	% ..	% 17	% 0	% 17	% 3	% 0	% 3	% 7	% 0	% 7
46-60	63	19	82	33	6	39	39	5	44	16	0	16	..	..	..	20	0	20	6	0	6	9	0	9
61 and over	74	26	100	50	5	55	41	12	53	27	0	27	..	..	..	13	0	13	24	5	29	16	0	16
Total	65	21	86	38	5	43	36	6	42	19	0	19	17	0	17	15	0	15	11	2	13	12	0	12

## Sex Groups

Male	63	23	86	43	6	49	40	7	47	18	0	18	..	..	..	12	0	12	10	2	14	12	0	12
Female	68	17	85	32	2	34	31	4	35	20	0	20	..	..	..	21	0	21	13	2	15	10	0	10

## Racial Groups

White	65	20	85	37	7	44	33	6	39	21	0	21	..	..	..	13	0	13	10	2	14	13	0	13
Black	66	22	88	40	0	40	40	5	45	15	0	15	..	..	..	18	0	18	17	4	12	10	0	10

\* Brain examined in 29 cases only.

included in the control group most cases of rheumatic heart disease were automatically excluded.

*Distribution of Arteriolar Sclerosis:* An examination of the total incidence of arteriolar sclerosis in the various tissues of non-hypertensives showed a high incidence in the spleen (86 per cent), pancreas (43 per cent) and adrenal capsules (42 per cent). Of all the tissues recorded in Table I, the renal arterioles were found to be least frequently diseased. There were 12 instances of renal arteriolar sclerosis in the entire control group, and of these, the sclerosis was classified as mild in 10 and moderately severe in 2.

### *Occurrence of Various Histological Types of Chronic Arteriolar Changes in Non-Hypertensive Individuals*

*Intimal Hyalinization:* All three anatomical types of chronic arteriolar disease were seen. The most common was intimal hyalinization. In the control group this was more pronounced in vessels over 50  $\mu$  in diameter. It was found most commonly in the spleen, pancreas, the capsules of the adrenals, brain, eye and kidney. It occurred in vessels of the internal genitalia and in the hyperplastic intima of arterioles in regions the seat of chronic or healed inflammation. It was rarely found in the gastro-intestinal tract except in association with inflammation, in liver, skeletal muscle, subcutaneous tissue, lymph nodes or bone marrow.

*Medial Hypertrophy and Degeneration:* Medial hypertrophy and degeneration were seen in the gastro-intestinal tract in 19 per cent and in the skeletal muscle of 15 per cent of the non-hypertensives. In no instance was it graded as a severe change. This type of arteriolar disease was seen frequently in the spleen but was usually obscured by the more striking intimal disease.

*Endothelial Hyperplasia:* Intimal endothelial hyperplasia in the arterioles of the non-hypertensive group was seen principally in association with involutional or inflammatory changes. It was seen almost constantly in the ovary, uterus and Fallopian tubes. It was seen in the adventitial arterioles in syphilitic aortitis and in association with chronic peptic ulcer, cholecystitis and appendicitis and a variety of other chronic inflammatory conditions. Medial degeneration in the form of intercellular collagen as well as intimal hyalinization were frequently associated changes.

*Age and Sex Differences:* Table I shows the relation of age to the incidence and severity of arteriolar sclerosis in the non-hypertensive group. In practically all of the tissues examined the arteriolar lesions were seen with greater frequency and severity as age advanced. There were no consistent differences between males and females or between blacks and whites in regard to incidence or severity of arteriolar sclerosis.

### PATHOLOGICAL CHANGES IN ARTERIOLES OF HYPERTENSIVE INDIVIDUALS

The incidence and severity of arteriolar sclerosis in the various organs and tissues of the hypertensive group are shown in Table II. As in the case of Table I, the internal genitalia are not included because of the common occurrence of vascular changes associated with cyclic involution and hyperplasia. The heart and lungs were omitted because of the rarity of chronic arteriolar changes in these situations in hypertensives except in association with rheumatic heart disease.

*Distribution and Severity of Arteriolar Lesions:* An examination of the total incidence of arteriolar sclerosis in the various tissues shows approximately the same frequency in the spleen and the kidney, 98 per cent and 97 per cent respectively. In the hypertensive group there was approximately the same sequence of organs, according to the incidence of arteriolar disease, except for the kidney, which changed from being one of the organs least frequently the seat of arteriolar sclerosis in the non-hypertensives to sharing first place with the spleen in being most frequently the seat of arteriolar sclerosis. In every organ and tissue, however, there was an increase in both frequency and severity of arteriolar disease throughout the hypertensive group as compared to the controls.

In grading, arteriolar sclerosis was designated as mild, moderate or severe. In the hypertensive group, severe arteriolar sclerosis was observed in the kidneys (47 per cent), gastro-intestinal tract (15 per cent), skeletal muscle (14 per cent), liver (11 per cent) and brain (8 per cent), in contrast with the non-occurrence of severe arteriolar sclerosis in any of those situations in members of the control group. Although severe vascular lesions were ob-

TABLE II

*The Relation of Age, Sex and Race to the Incidence and Severity of Arteriol Sclerosis in Hypertensive Individuals*

Age Groups	Spleen			Pancreas			Adrenal			Gastro-Intestinal			Brain *			Skeletal muscle			Liver			Kidney		
	Mild or moderate	Severe	Total	Mild or moderate	Severe	Total	Mild or moderate	Severe	Total	Mild or moderate	Severe	Total	Mild or moderate	Severe	Total	Mild or moderate	Severe	Total	Mild or moderate	Severe	Total	Mild or moderate	Severe	Total
	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%
31-45 yrs.	46	54	100	21	52	73	51	39	90	44	20	64	..	..	..	64	17	81	38	9	47	33	67	100
46-60	39	61	100	28	62	90	56	38	94	64	15	79	..	..	..	62	25	87	36	19	55	46	50	96
61 and over	44	52	96	56	31	87	50	44	94	48	7	55	..	..	..	48	3	51	41	6	47	72	22	94
Total	44	55	99	40	47	87	51	42	93	55	15	70	42	8	50	58	14	72	38	11	49	50	47	97

Sex Groups	Spleen			Pancreas			Adrenal			Gastro-Intestinal			Brain *			Skeletal muscle			Liver			Kidney		
	Mild or moderate	Severe	Total	Mild or moderate	Severe	Total	Mild or moderate	Severe	Total	Mild or moderate	Severe	Total	Mild or moderate	Severe	Total	Mild or moderate	Severe	Total	Mild or moderate	Severe	Total	Mild or moderate	Severe	Total
	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%
Male	50	50	100	42	42	84	51	37	88	60	10	70	..	..	..	58	12	70	36	7	43	47	51	98
Female	37	60	97	38	53	91	51	49	100	50	20	70	..	..	..	62	16	78	40	18	58	54	41	95

Racial Groups	Spleen			Pancreas			Adrenal			Gastro-Intestinal			Brain *			Skeletal muscle			Liver			Kidney		
	Mild or moderate	Severe	Total	Mild or moderate	Severe	Total	Mild or moderate	Severe	Total	Mild or moderate	Severe	Total	Mild or moderate	Severe	Total	Mild or moderate	Severe	Total	Mild or moderate	Severe	Total	Mild or moderate	Severe	Total
	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%
White	45	55	100	38	48	86	52	42	94	55	19	74	..	..	..	55	18	73	33	15	48	45	51	96
Black	42	54	96	46	43	89	48	42	90	55	7	62	..	..	..	68	4	72	48	3	51	59	41	100

\* Brain examined in 33 cases only.



served in the spleen, pancreas and capsules of adrenals of some of the controls, the incidence of severe arteriolar sclerosis was more than doubled in those same organs in the hypertensive group.

*Age and Sex Differences:* In contrast with the control group in which the occurrence and severity of vascular disease increased with age, the general trend in the hypertensive group was for vascular disease to be less severe in the older individuals. An especially striking drop in the severity of arteriolar sclerosis with age was seen in the kidney, where the vascular disease was severe in 67 per cent of all hypertensives dead under the age of 45, in 50 per cent of hypertensives dead between the ages of 46 and 60, and in only 22 per cent of hypertensives dead after the age of 61 years.

No significant differences in the occurrence or severity of arteriolar disease could be seen in males and females or in blacks and whites within the hypertensive group.

### *Occurrence of Various Histological Types of Arteriolar Sclerosis in Hypertensive Individuals*

*Endothelial Hyperplasia:* In a discussion of the anatomical types of arteriolar disease seen in the various tissues in the hypertensive group the occurrence of intimal proliferation not demonstrably caused by inflammation of surrounding tissues appeared to deserve first consideration. This type of change was most pronounced in renal vessels over 30  $\mu$  in diameter, giving way to intimal hyalinization in the afferent arterioles. It appeared frequently to be associated with medial degeneration (intercellular collagen and thinning), and in many instances was complicated by hyaline, fat, mucoid or chromatropic degeneration, or necrosis of the hyperplastic intima as well as of the media. Although primary intimal hyperplasia was observed most frequently in the kidneys, it was seen occasionally in a variety of other situations. In no instance was it encountered in the skeletal muscle, subcutaneous tissue, lymph nodes or bone marrow, except in association with obvious local inflammation.

*Acute Arteriolar Lesions:* Acute degenerative, necrosing and inflammatory lesions were frequently superimposed on chronic arteriolar lesions of all kinds and were seen most frequently (38

per cent of all cases of chronic hypertension) in the renal arterioles, especially those showing intimal hyperplasia. The lesions varied greatly as to character and severity and were so obviously secondary that although they may have been significant in relation to the rapidity with which the disease progressed, they were not considered significant in relation to the inception of either the hypertension or the renal vascular disease. No tissue or organ was immune from the occurrence of these vascular lesions and, next to the kidney, they were seen most frequently in the gastro-intestinal tract.

*Intimal Hyalinization:* Intimal hyalinization occurred in the same tissues in the hypertensive as in the non-hypertensive group but with greater frequency and severity in the former. It was seen commonly as a generalized vascular disease in such tissues as the kidney, gastro-intestinal tract, liver and brain, where its occurrence in any degree of severity in non-hypertensives was rare. In the kidney and eye it was frequently associated with endothelial hyperplasia in larger arterioles (50 to 100  $\mu$  in external diameter). There was no qualitative difference in the intimal hyalin in the arterioles of the hypertensive and the non-hypertensive groups, although secondary degenerative changes were more common in the former.

*Medial Hypertrophy and Degeneration:* Medial hypertrophy and collagenous degeneration were widespread and common types of arteriolar changes in the hypertensive group. Without complicating intimal disease these changes were seen most frequently in the skeletal muscle. In a number of instances in which samples of more than one skeletal muscle from the same individual were examined, there appeared to be no significant difference in the severity of the vascular disease in various portions of the skeletal muscular system, with the possible exception of the diaphragm. Sections were prepared from the pectoralis major, rectus abdominis, quadriceps femoris, psoas and diaphragm from 6 individuals having severe generalized arteriolar sclerosis. With the exception of the diaphragm, the various muscles exhibited, as nearly as could be judged, the same degree of arteriolar sclerosis. In 3 of the 6, the diaphragmatic arterioles either appeared normal or mildly sclerotic, whereas in other muscles moderate or severe arteriolar sclerosis was present.

## MEASUREMENTS OF WALL THICKNESS OF ARTERIOLES

The claims of Keith, Barker and Kernohan to the effect that the thickening of the walls of the arterioles of skeletal muscle, due principally to medial hypertrophy, could be demonstrated by measurements in microscopic sections, deserved corroboration if their assumptions were correct. Their claims were made on a basis of measuring several arterioles from each of 50 control cases and from 143 cases of chronic hypertension. They found that arteriolar thickening was frequently present in hypertensive individuals and described five different types of chronic hypertension in which different mean wall to lumen ratios were observed. They did not state what the error of the method was or what the observed anatomical variations were. Bell, as well as Andrus, has denied the probability of medial hypertrophy of arterioles in the skeletal muscle of chronic hypertensives, but neither has presented quantitative data to refute the findings of Keith, Barker and Kernohan.

*Variations in Measurements of Arterioles Not Related to Disease*

To determine the extent of the variation in arteriolar measurements incident to errors in mensuration or to differences in relative wall thickness related to different degrees of postmortem contraction or to actual anatomical differences, 15 samples of 75 arterioles each from the pectoral muscle of 1 non-hypertensive individual were measured and the mean external diameter, the mean lumen diameter and the mean ratio of wall thickness to lumen diameter were calculated. The 15 samples were fixed and prepared for microscopic examination by a uniform technic. The vessels were measured by the method described by Kernohan, Anderson and Keith, using an 8 mm. objective and a X 7 ocular containing a micrometer scale. Every arteriole between 18 and 72  $\mu$  in diameter was measured as it was encountered in cross section until 75 vessels had been measured. The outermost cells of the media were used to define the external diameter. To avoid measuring the same arteriole in succeeding sections, the sections from each block of muscle were cut at intervals of between 0.5 and 1.0 mm.

The means of the arteriolar measurements of the 15 samples of 75 arterioles each are given in Table III. This record indicates that the least variable mean was that of the external diameter of

a sample of arterioles having a fixed size range. In this instance it was  $31.9 \mu$  with a probable error of  $1.5 \mu$ . The variation of the wall to lumen ratio was great, ranging from 1.0-1.4 to 1.0-2.5. It is apparent then that any difference in the wall to lumen ratio within the limits of 1.0-1.4 and 1.0-2.5 in 2 samples of 75 arterioles each, or less, would have no significance unless some more accurate method of measurement were employed than was used in

TABLE III

*Record of Measurements of 15 Samples With 75 Arterioles in Each From the Pectoral Muscle of 1 Non-Hypertensive Individual*

Sample	Mean external diameter	P.E.m.	Mean internal diameter	P.E.m.	Wall to lumen ratio
	$\mu$		$\mu$		
1	33.4	1.04	16.0	0.58	1 to 1.8
2	31.4	1.08	17.4	0.76	1 to 2.5
3	32.2	0.90	15.5	0.54	1 to 1.9
4	33.8	0.97	17.9	0.72	1 to 2.3
5	31.4	0.83	15.3	0.36	1 to 1.9
6	34.0	1.30	17.1	0.72	1 to 2.0
7	30.1	1.01	15.2	0.54	1 to 2.0
8	32.5	1.08	16.7	0.72	1 to 2.1
9	28.8	0.97	13.8	0.54	1 to 1.8
10	29.4	0.90	15.0	0.54	1 to 2.1
11	30.2	0.97	15.0	0.76	1 to 2.0
12	31.1	1.01	16.1	0.65	1 to 2.0
13	31.8	0.97	15.8	0.61	1 to 2.0
14	30.7	0.86	17.1	0.58	1 to 2.5
15	30.1	1.04	15.9	0.61	1 to 2.2

Mean external diameter =  $31.4 \mu$  P.E.  $\pm 1.5$

Mean internal diameter =  $16.0 \mu$  P.E.  $\pm 1.0$

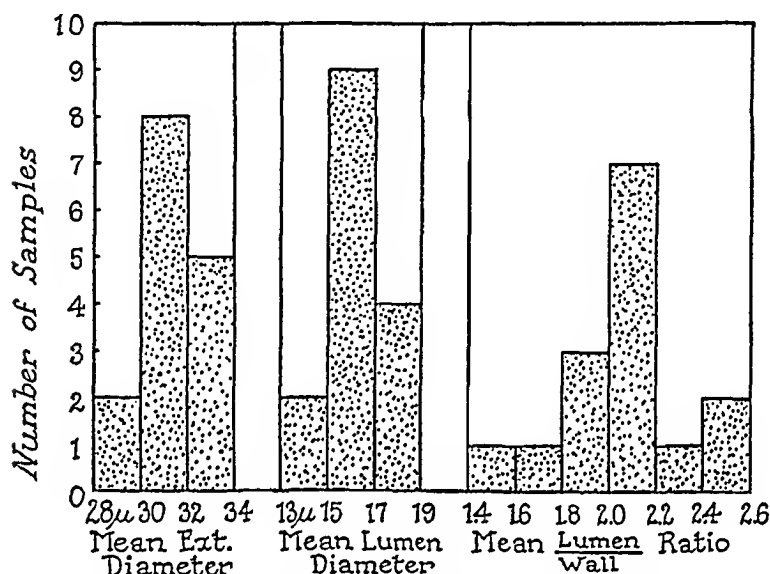
Mean wall to lumen ratio = 1 to 2.0

this investigation. With such variation, it hardly seemed likely that the differences in the first decimal between mean ratios of wall thickness to lumen diameter in samples of undefined magnitude, such as reported by Keith, Barker and Kernohan, were significant. The distribution of each of the three means for the 15 samples is shown in Text-Fig. 3.

#### *Selection of Control and Hypertensive Cases for Arteriolar Measurements*

It was felt from this test of multiple samples from 1 individual that many samples of arterioles from the skeletal muscle of hyper-

tensives and non-hypertensives would have to be measured in order to determine whether real differences in relative wall thickness exist or not. Accordingly, 38 classical cases of essential hypertension, with death from renal insufficiency, heart failure or cerebral hemorrhage, were selected for study. These were about equally divided between males and females, between blacks and



Text Fig. 3. Histograms showing the distribution of 15 samples of pectoral muscle from one non-hypertensive individual according to the mean external diameter, the mean lumen diameter and the mean wall to lumen ratio of arterioles in each sample. Seventy five arterioles ranging between 18 and 72  $\mu$  in external diameter constitute a sample.

whites, and between persons over and under 45 years of age. A control group of non-hypertensive individuals with comparable age, sex, and race population was selected. As in the case of previously reported measurements, the external and internal diameter of each of 75 arterioles within the limits of 18 and 72  $\mu$  in external diameter in samples of pectoral muscle from each of the 76 individuals were measured. These data are shown in Table IV.

TABLE IV

*Record of Measurements of 75 Arterioles From the Pectoral Muscle of Each of 38 Non-Hypertensive and 38 Hypertensive Individuals*

Non-hypertensive individuals						Hypertensive individuals						
Age	Sex	Color	E.D.	I.D.	Ratio	Age	Sex	Color	Cause of death	E.D.	I.D.	Ratio
yrs.			$\mu$	$\mu$		yrs.				$\mu$	$\mu$	
43	F	W	31.7	15.1	1 to 1.7	37	M	B	R	38.2	11.5	1 to 0.9
32	F	B	29.2	11.9	1 to 1.4	59	M	W	H	41.8	15.8	1 to 1.2
41	M	W	33.8	18.0	1 to 2.3	50	F	B	H	33.8	14.8	1 to 1.5
46	F	W	32.4	16.2	1 to 2.0	43	F	B	R	37.8	16.2	1 to 1.5
48	F	W	30.6	11.9	1 to 1.2	50	M	B	H	36.7	14.4	1 to 1.3
53	M	W	34.2	19.1	1 to 2.5	54	M	B	C	44.3	19.1	1 to 1.6
38	M	B	34.9	19.8	1 to 2.5	38	F	W	C	46.1	14.0	1 to 0.9
36	F	W	34.2	16.6	1 to 1.9	50	M	B	C	39.2	10.4	1 to 1.1
36	F	W	36.4	21.6	1 to 2.9	38	F	W	H	31.3	13.7	1 to 1.6
48	F	W	30.2	15.1	1 to 2.0	49	F	B	H	33.5	14.4	1 to 1.5
38	M	W	31.3	15.5	1 to 2.0	58	M	B	R	41.0	16.2	1 to 1.3
50	M	W	34.9	18.7	1 to 2.3	54	F	B	H	34.2	12.6	1 to 1.2
57	M	W	30.2	15.5	1 to 2.1	33	F	B	R	34.6	14.8	1 to 1.5
52	M	W	36.4	18.0	1 to 2.0	40	F	B	H	36.7	17.6	1 to 1.9
40	M	W	31.3	16.6	1 to 2.3	58	M	W	H	39.2	20.2	1 to 2.1

E.D. = Mean external diameter of 75 arterioles varying from 18 to 72  $\mu$  in external diameter.

I.D. = Mean internal diameter of 75 arterioles.

Ratio = Unweighted ratio of mean wall thickness to mean lumen diameter of 75 arterioles.

Cause of death: H = heart failure.

R = renal failure.

C = cerebral hemorrhage.

TABLE IV — Continued

Non-hypertensive individuals						Hypertensive individuals						
Age	Sex	Color	E.D.	I.D.	Ratio	Age	Sex	Color	Cause of death	E.D.	I.D.	Ratio
yrs.			$\mu$	$\mu$		yrs.				$\mu$	$\mu$	
57	M	W	32.8	13.0	1 to 1.3	58	M	W	H	34.2	13.3	1 to 1.3
36	F	W	33.1	16.2	1 to 1.9	50	F	B	C	33.8	16.2	1 to 1.8
62	M	W	30.2	11.5	1 to 1.2	41	M	B	H	39.2	13.3	1 to 1.0
49	M	W	30.2	15.8	1 to 2.2	51	F	B	R	38.6	16.9	1 to 1.5
58	F	W	32.8	16.9	1 to 2.1	40	M	W	H	37.1	17.6	1 to 1.8
34	M	B	30.6	15.1	1 to 1.9	40	F	B	C	36.4	18.4	1 to 2.0
41	M	W	36.0	16.2	1 to 1.6	37	F	W	R	41.8	14.8	1 to 1.1
44	M	W	30.2	16.2	1 to 2.3	36	M	B	R	36.4	14.4	1 to 1.3
55	F	W	32.8	13.7	1 to 1.4	34	M	B	H	36.0	16.2	1 to 1.6
49	F	W	32.0	16.9	1 to 2.2	36	M	W	C	32.8	10.8	1 to 1.0
37	F	W	29.2	14.0	1 to 1.8	55	M	W	H	43.6	20.2	1 to 1.7
54	M	W	30.6	14.4	1 to 1.9	50	F	B	R	34.2	15.1	1 to 1.6
58	F	W	36.0	18.7	1 to 2.2	44	M	W	R	41.8	13.3	1 to 0.9
34	M	B	32.4	16.9	1 to 2.2	45	M	W	R	46.1	17.6	1 to 1.2
34	M	B	33.1	16.6	1 to 2.0	37	F	W	R	42.5	15.5	1 to 1.1
45	M	W	33.5	17.6	1 to 2.2	36	F	B	R	42.1	15.5	1 to 1.2
50	M	W	29.9	11.9	1 to 1.3	31	M	W	R	36.4	13.0	1 to 1.1
35	F	W	28.1	13.3	1 to 1.8	48	F	W	R	47.2	14.8	1 to 0.9
38	F	B	32.4	16.6	1 to 2.1	36	F	W	H	36.7	15.1	1 to 1.4
38	F	B	29.5	12.6	1 to 1.5	58	F	B	C	33.8	16.2	1 to 1.8
52	M	W	33.5	17.3	1 to 2.1	51	M	W	H	39.6	20.5	1 to 2.1
59	M	B	29.2	13.0	1 to 1.6	53	M	W	H	35.6	13.3	1 to 1.2
40	M	B	33.8	16.2	1 to 1.8	45	F	W	H	44.6	16.6	1 to 1.2

*Comparison of Arteriolar Measurements in Control and Hypertensive Groups*

A comparison of the means of the mean external diameters for each group (hypertensives and non-hypertensives) shows:

32.2 P.E.  $\pm$  2.2  $\mu$  for the controls

and

38.4 P.E.  $\pm$  4.1  $\mu$  for the hypertensives

The difference is significant.

A comparison of the means of the mean lumen diameters for each group shows:

15.8 P.E.  $\pm$  2.3  $\mu$  for the controls

and

15.4 P.E.  $\pm$  2.4  $\mu$  for the hypertensives

The difference is not significant.

A comparison of the means of the mean wall to lumen ratio shows:

1.0-1.9 for the controls

and

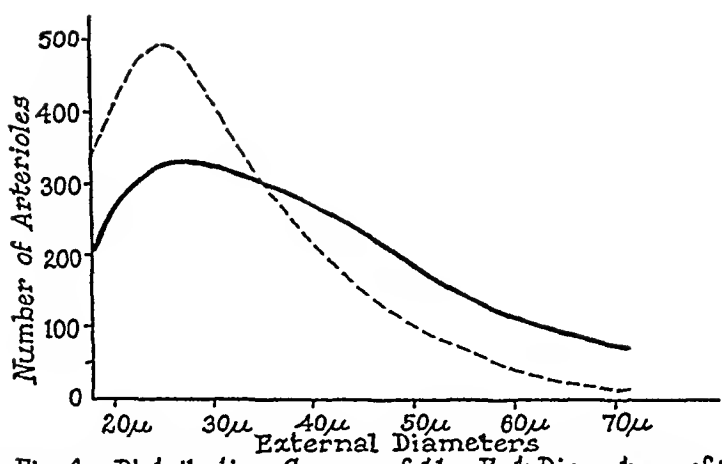
1.0-1.36 for the hypertensives.

The difference is significant.

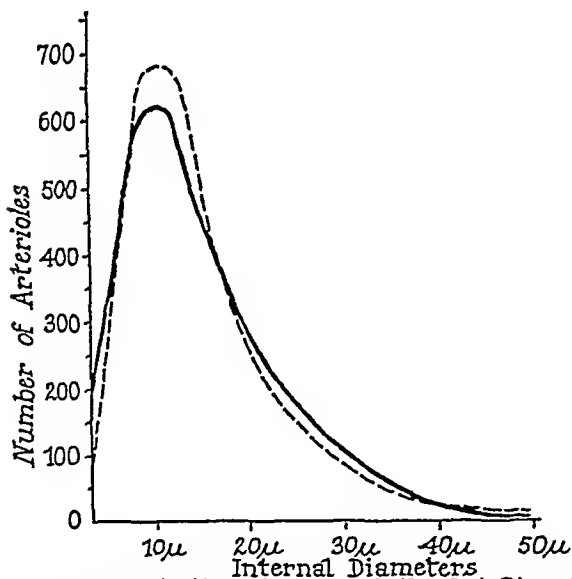
So far as the means of the various measurements of the arterioles of the hypertensive and the non-hypertensive groups are concerned, it appeared that the arterioles of the pectoral muscle of hypertensive individuals have relatively thicker walls than those of the non-hypertensives, that the arterioles are larger, as indicated by the mean external diameter, but that there is no real evidence that the arteriolar lumens of the hypertensives have been reduced.

A comparison of the external and internal diameters of arterioles in hypertensive and non-hypertensive individuals is shown in Text-Figs. 4 and 5. In Text-Fig. 4 the external diameters of 2850 arterioles of hypertensives and a like number of arterioles from non-hypertensives have been plotted against the number of vessels in each size group to establish two distribution curves. It may be seen that there are many more arterioles in the small vessel group





Text Fig. 4. Distribution Curves of the Ext. Diameters of:  
— 2850 arterioles in pectoral muscle of hypertensive individuals.  
--- 2850 " " " " " non- " "



Text Fig. 5. Distribution Curves of the Int. Diameters of:  
— 2850 arterioles in pectoral muscle of hypertensive individuals.  
--- 2850 " " " " " non- " "

75 arterioles ranging from 18 to 72μ in external diameter from each of 38 hypertensive and 38 non-hypertensive individuals, were measured.

(18 to 36  $\mu$  in external diameter) in the controls than in the hypertensives, and that over an external diameter of 36  $\mu$ , vessels are more numerous in the hypertensive groups. This flattening of the curve represents an increase in the mean external diameter of arterioles in the hypertensive group.

In Text-Fig. 5 the internal diameters of the same arterioles have been plotted as abscissae against the number of vessels in each size group as ordinates. There is practically no difference in the two curves, which indicates that in so far as the two groups of cases (hypertensives and non-hypertensives) are concerned, the internal diameters of arterioles in the pectoral muscles are not significantly different.

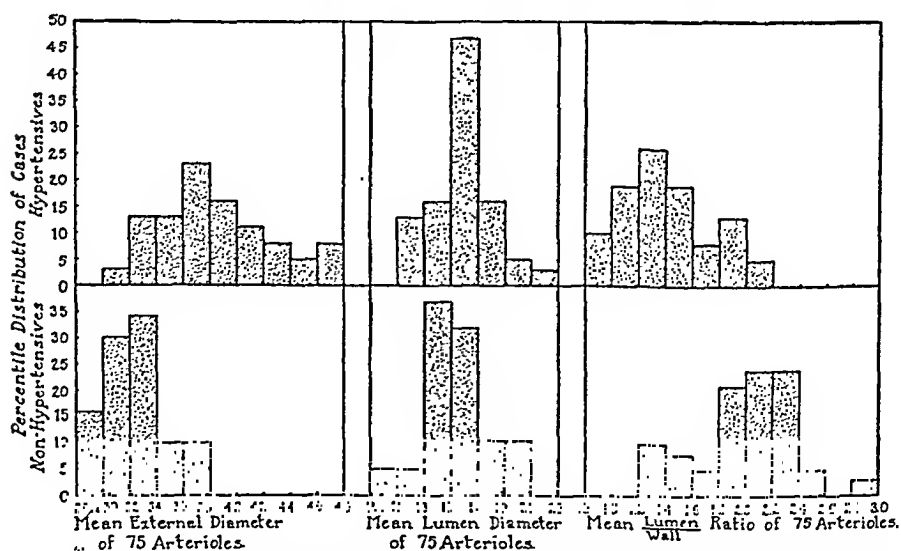
No deductions can be drawn as to the relative patency of the two groups of arterioles in life. It is possible that medial degeneration rendered the arterioles in the pectoral muscle of hypertensives relatively resistant to postmortem contraction, so that although they appeared to have the same degree of patency as the more fully contracted normal arterioles in tissue sections, they may have had narrower lumens than normal vessels in life. It is equally possible that they were more fully contracted than normal arterioles and that the increased wall thickness in death is a reflection of a relatively greater lumen diameter in life. Differences in the functional state of arterioles in the skeletal muscle of hypertensive and non-hypertensive individuals cannot be conclusively determined from differences in measurements of postmortem material.

Table V shows the results of the arteriolar measurements in the various subgroups of the 38 hypertensive individuals. It is seen that so far as mean internal and external diameter, and mean wall to lumen ratio are concerned, there are no significant differences between individuals under and over 45 years of age, between males and females, blacks and whites, or between those dead of uremia, heart failure or cerebral hemorrhage.

Finally, in this consideration of the measurements of arterioles, attention was turned to the possible value of measuring arterioles in samples of pectoral muscle to distinguish between hypertensive and non-hypertensive individuals. Text-Fig. 6 was prepared to show the correlation of mean external and lumen diameter values, as well as mean ratios of wall to lumen thickness with chronic hypertension.

TABLE V  
*Hypertensive Group*  
*Skeletal Muscle*

	No. of cases	Age	E.D.	I.D.	Wall to lumen ratio
Entire hypertensive group	38	yrs. 43.8	$\mu$ 38.4	$\mu$ 15.4	1 to 1.3
31 to 45 years of age	20	38.4	38.7	15.0	1 to 1.3
46 to 60 years of age	18	49.7	37.6	15.8	1 to 1.4
Male	19	43.7	38.2	15.4	1 to 1.4
Female	19	43.9	37.9	15.4	1 to 1.4
White	18	41.7	39.9	15.6	1 to 1.3
Black	20	45.7	37.0	15.2	1 to 1.4
Death from heart failure	17	46.6	37.2	15.5	1 to 1.4
Death from renal insufficiency	14	41.9	39.9	15.0	1 to 1.2
Death from cerebral hemorrhage	7	46.6	38.1	15.0	1 to 1.3



Text Fig. 6. Histograms showing the percentile distribution of 38 control and 38 hypertensive cases, according to the mean external diameter, the mean lumen diameter and the mean wall to lumen ratio of arterioles in samples of pectoral muscle from each. Seventy five arterioles ranging between 18 and 72 $\mu$  in external diameter constitute a sample.

It was found that in 48 per cent of the hypertensives the mean external diameter of the 75 vessel samples was greater than that found in any of the control group. If the control cases were representative normals it might be said that whenever the mean external diameter of a sample of 75 arterioles, ranging between 18 and 72  $\mu$  in external diameter, exceeded 38  $\mu$  the sample was from a chronic hypertensive individual. Moreover, in almost half of the group of the chronic hypertensives the mean external diameter was greater than 38  $\mu$ . The correlation of mean external diameter and a state of normal blood pressure was less striking. Sixteen per cent of the non-hypertensives had mean external diameters lower than those seen in any hypertensive. So far as these data were representative, it appeared that when the mean external diameter fell below 30  $\mu$  chronic hypertension could be excluded.

There was no significant difference between the contour of the distribution curves or the means of the internal diameters of the same 75 vessel samples of pectoral muscle of the hypertensive and non-hypertensive groups.

It has already been stated that the mean wall to lumen ratio of these same samples of arterioles was 1.0–1.9 in the controls and 1.0–1.36 in the hypertensives. The distribution curves of these ratios (Text-Fig. 6) showed a wide overlapping with only 29 per cent of the hypertensive group having a wall to lumen ratio less than that seen in any of the controls. It appeared that if the wall to lumen ratio of a sample of arterioles, such as were measured in this study, was less than 1.0–1.2, that sample was invariably from a hypertensive individual. The distribution curves showed that if the wall to lumen ratio was greater than 1.0–2.2, chronic hypertension could be excluded. Hypertension could be excluded in 32 per cent of the normals by this criterion.

#### *Variations in Arteriolar Measurements Related to Differences in Methods of Tissue Preparation*

It was thought that differences in the time at which tissues were fixed in relation to death, differences in the duration of fixation, dehydration and clearing and differences in the time in the paraffin oven might lead to measurable differences in the dimensions of arterioles. To determine whether this were true or not, 8 samples

of pectoral muscle obtained from 1 non-hypertensive individual were prepared for histological examination by the various methods indicated in Table VI. Unless otherwise indicated the tissue was fixed during rigor and kept in 10 per cent formalin for 24 hours. The standard time allowed for dehydration and clearing was 12 hours and for embedding (paraffin oven) 3 hours. All sections were cut at a thickness of  $6\ \mu$  and stained in the same manner. Seventy-five arterioles ranging from 18 to  $72\ \mu$  in external diameter were measured in each sample.

Table VI shows that there were no differences between the means greater than the differences observed in samples subjected

TABLE VI

*A Comparison of the Mean External Diameter, Mean Internal Diameter, and Mean Wall to Lumen Ratio of Arterioles in 8 Samples (75 Arterioles Between 18 and  $72\ \mu$  in External Diameter in Each Sample) From the Pectoral Muscle of 1 Non-Hypertensive Individual, to Show the Effect of Variations in Methods of Tissue Preparation On the Dimensions of Arterioles*

Sample	Treatment	Mean external diameter	Mean internal diameter	Wall to lumen ratio
1	Fixed before onset of rigor	$\mu$ 34.9	$\mu$ 17.7	1 to 2.1
2	Fixed during rigor	31.6	16.2	1 to 2.1
3	Fixed after dissipation of rigor	32.8	15.8	1 to 1.9
4	Fixed for 168 hours	36.0	20.3	1 to 2.6
5	Dehydration and clearing for 6 hours	31.5	15.1	1 to 1.8
6	Dehydration and clearing for 62 hours	31.5	14.4	1 to 1.7
7	In paraffin oven for $1\frac{1}{2}$ hours	32.3	15.2	1 to 1.8
8	In paraffin oven for 24 hours	34.6	18.3	1 to 2.2

to a uniform method of tissue preparation (see Table III and Text-Fig. 3.) Although this experiment does not prove that variations in technic do not influence the size of vessels in microscopic preparations, yet it does indicate that the anatomical variations and errors in mensuration under controlled conditions account for as great differences as result from deliberate variations in methods of tissue preparation.

*Variations in Arteriolar Measurements Related to Differences in the Physiological State of the Vascular System*

It was thought that differences in the functional state of arterioles (vasoconstriction or vasodilatation) at the time of death or at the time that the biopsy was taken might be reflected in differences in relative wall thickness in microscopic preparations. To determine whether this were true or not the following experiments were conducted on 4 dogs.

The dogs were mongrels, ranged from 8 to 12 kg. in weight, were mature but were of unknown ages. Each animal was anesthetized with ether and a carotid canula was inserted and connected with a mercury manometer and the systolic blood pressure recorded on a kymograph. As soon as the blood pressure had reached a fairly constant plateau, a biopsy of skeletal muscle was taken. Following this biopsy, the injection of 0.5 cc. per kilo of 1:1000 adrenalin was followed by a rise in blood pressure, at the peak of which another sample of skeletal muscle was taken. After waiting for 30 minutes, 0.5 cc. per kg. of a 1:1000 solution of nitroglycerin was injected intravenously and when the blood pressure had fallen below the original resting level the last biopsy of skeletal muscle was taken. All specimens were fixed for the same period and prepared for histological examination in the same manner. Sections were cut at intervals of about 0.5 mm. from each block of tissue and 50 arterioles ranging from 18 to 72  $\mu$  in external diameter were measured in each specimen. The results of these measurements are recorded in Table VII.

To determine whether the observed differences were significant or not, 7 samples of skeletal muscle were taken at the same time from the shoulder of 1 dog and the samples were prepared for microscopic examination in the same manner. Fifty arterioles (18 to 72  $\mu$  in external diameter) in each sample were measured as in the preceding experiment and the measurements were recorded in Table VIII. It was seen that the variations in mean external diameter, mean internal diameter and mean wall to lumen ratio, associated with differences in the physiological state of the vascular system, were no greater than those observed in the samples taken under uniform physiological conditions. Although this experiment does not prove that variations in the

TABLE VII

*A Comparison of the Mean External Diameter, the Mean Internal Diameter and the Mean Wall to Lumen Ratio of Arterioles in Samples of Skeletal Muscle (50 Arterioles Ranging Between 18 and 72  $\mu$  in External Diameter in Each Sample) in Different States of Arterial Hyper- and Hypotension from Each of 4 Dogs*

Dog No.	Experimental conditions	Systolic blood pressure	Source of muscle	Mean external diameter of 50 arterioles $\mu$	Mean internal diameter of 50 arterioles $\mu$	Mean wall to lumen ratio of 50 arterioles
1	Resting After adrenalin After nitroglycerin	<i>mm. Hg.</i> 160 to 180	Shoulder	32.4	14.8	1 to 1.7
		270	"	35.3	15.5	1 to 1.6
		140	"	37.6	17.3	1 to 1.7
2	Resting After adrenalin After nitroglycerin	150 to 170	"	29.5	15.8	1 to 2.3
		240	"	29.2	15.1	1 to 2.1
		120	"	29.9	15.1	1 to 2.0
3	Resting After adrenalin After nitroglycerin	160 to 170	Thigh	29.3	10.3	1 to 1.1
		300 +	"	27.7	8.4	1 to 0.9
		150	"	27.4	10.3	1 to 1.2
4	Resting After adrenalin After nitroglycerin	160 to 170	"	28.1	10.6	1 to 1.2
		280	"	27.4	10.6	1 to 1.3
		140	"	31.9	12.3	1 to 1.3

physiological state of vessels do not influence their size in microscopic preparations, yet it does indicate that anatomical variations, together with errors in mensuration under controlled conditions, account for as great differences as were observed in varying physiological states.

TABLE VIII

*A Comparison of the Mean External Diameter, Mean Internal Diameter and Mean Wall to Lumen Ratio of Arterioles in Each of 7 Samples (50 Arterioles Ranging Between 18 and 72  $\mu$  in External Diameter in Each Sample) Taken at the Same Time and Subjected to the Same Methods of Tissue Preparation From the Shoulder Muscle of a Dog*

Sample	Mean external diameter	Mean internal diameter	Mean wall to lumen ratio
1	$\mu$ 30.2	$\mu$ 16.2	1 to 2.3
2	27.4	14.8	1 to 2.3
3	26.3	13.3	1 to 2.0
4	27.0	13.7	1 to 2.1
5	25.9	12.6	1 to 1.9
6	28.1	13.7	1 to 1.9
7	28.8	16.9	1 to 2.8

### PATHOGENESIS OF ARTERIOLAR SCLEROSIS

Impressions as to the pathogenesis of the various types of arteriolar sclerosis based on a statistical and morphological study of the disease were obviously inferential but certain relations and general trends deserve consideration.

Of perhaps the greatest significance is the fact that no type of chronic arteriolar disease was of and by itself pathognomonic of hypertension. Every type was found in persons known to have had normal blood pressures.

Intimal hyalinization appeared to be the arteriolar counterpart of simple arteriosclerosis (Plate 98), was essentially an aging phenomenon which developed precociously in some individuals and was seen most frequently in the abdominal organs supplied by relatively large short branches of the aorta (spleen, kidney,



pancreas and adrenals) as well as in the brain, spinal cord and eye. It was seen with greater frequency and severity in hypertensives than in non-hypertensives and in hypertensive individuals extended into vascular beds where its occurrence in non-hypertensives was very infrequent (liver and gastro-intestinal tract). Since intimal hyalinization appeared to be a simple "wear and tear" type of tissue reaction, and since it was reasonable to assume that chronic hypertension might augment arteriolar "wear and tear," it is not unreasonable to expect the lesion to be more widely distributed and more severe in hypertensives than in non-hypertensives. It is also possible that the arteriolar changes secondary to chronic hypertension frequently effect such widespread organic reduction in lumen caliber that the severity of the hypertension is increased, thus establishing a vicious circle. There was, however, no proof that hypertension initiated intimal hyalinization. The development of visible arteriolar disease in the retinal vessels in the course of essential hypertension and the later development of renal failure due to advanced nephrosclerosis do not necessarily indicate that these vascular changes are due to the high blood pressure. They may as well represent the further extension of a primary morbid process which causes hypertension. Neither does recovery from a state of chronic hypertension indicate that the disease is primarily functional rather than organic. Certainly organic vascular disease is not necessarily an irreversible process. The possibility that high blood pressure causes intimal hyalinization cannot be denied, but there is no conclusive evidence that such is the case and it is known that intimal hyalinization does occur quite independently of hypertension.

Another possible explanation for the increased severity of intimal hyalinization in hypertension is that only the more severe forms of arteriolar disease may in some way have been responsible for hypertension. Still another possible explanation is that the factors (humoral or reflex) that cause the arteriolar spasm that produces the increased peripheral resistance may also injure vessels with resulting intimal degeneration.

Intimal proliferation (Plate 101) was seen under three circumstances: (1) it was seen very frequently in association with chronic inflammation and apparently represented the characteristic vascular response to chronic inflammation in hypertensive

as well as non-hypertensive individuals; (2) it constituted a secondary adaptive change in arterioles supplying tissues that had undergone parenchymatous atrophy with a consequently diminished capillary bed and diminished blood flow (ovary and uterus); and (3) it was seen commonly as an independent and apparently primary morbid process most frequently in the kidney and occasionally in other tissues (pancreas, adrenal capsules, gall bladder, seminal vesicles, spleen, gastro-intestinal tract, and eyes of hypertensives.

The pathogenesis of the intimal hyperplasia in the first two of the above considered conditions seems to be primary exudative or non-exudative arteriolitis or arteriolar involution. The third consideration is best exemplified by the obliterating arteriolar intimal hyperplasia of nephrosclerosis in chronic hypertension and may be the result of inflammation, involution, neither or both. It is possible or even probable that this is a form of arteriolitis, occurring either as an augmented type of "wear and tear" arteriolar degeneration (Herxheimer) or as a primary independent arteriolar inflammation (Fahr). It is also possible that some or all of the arteriolar endothelial proliferation in the contracted kidney represents arteriolar involution secondary to obstruction proximal to it by spasm or distal to it by glomerular contraction (Volhard, Fishberg). It has been suggested by Moschcowitz that the endothelial proliferation is caused by the hypertension and in support of this theory he cites the endothelial hyperplasia of pulmonary arterioles in cases of mitral stenosis. The analogy is obviously not entirely applicable, inasmuch as in mitral stenosis there is, in addition to pulmonary arterial hypertension, stasis and cardiovascular inflammation.

Medial hypertrophy and degeneration uncomplicated by intimal thickening (Plates 99 and 100) were seen with greater frequency and severity in hypertensives than in non-hypertensives. The change appeared to be structurally analogous to the hypertrophy of any other hollow muscular structure. It is not known that arteriolar dilatation occurs in life as a result of high blood pressure, but it is presumed that arteriolar constriction does occur. Increased peripheral resistance to blood flow due to vasoconstriction is a *sine qua non* of chronic hypertension. Arteriolar dilatation is then the apparent antithesis of the vascular state of hypertension.

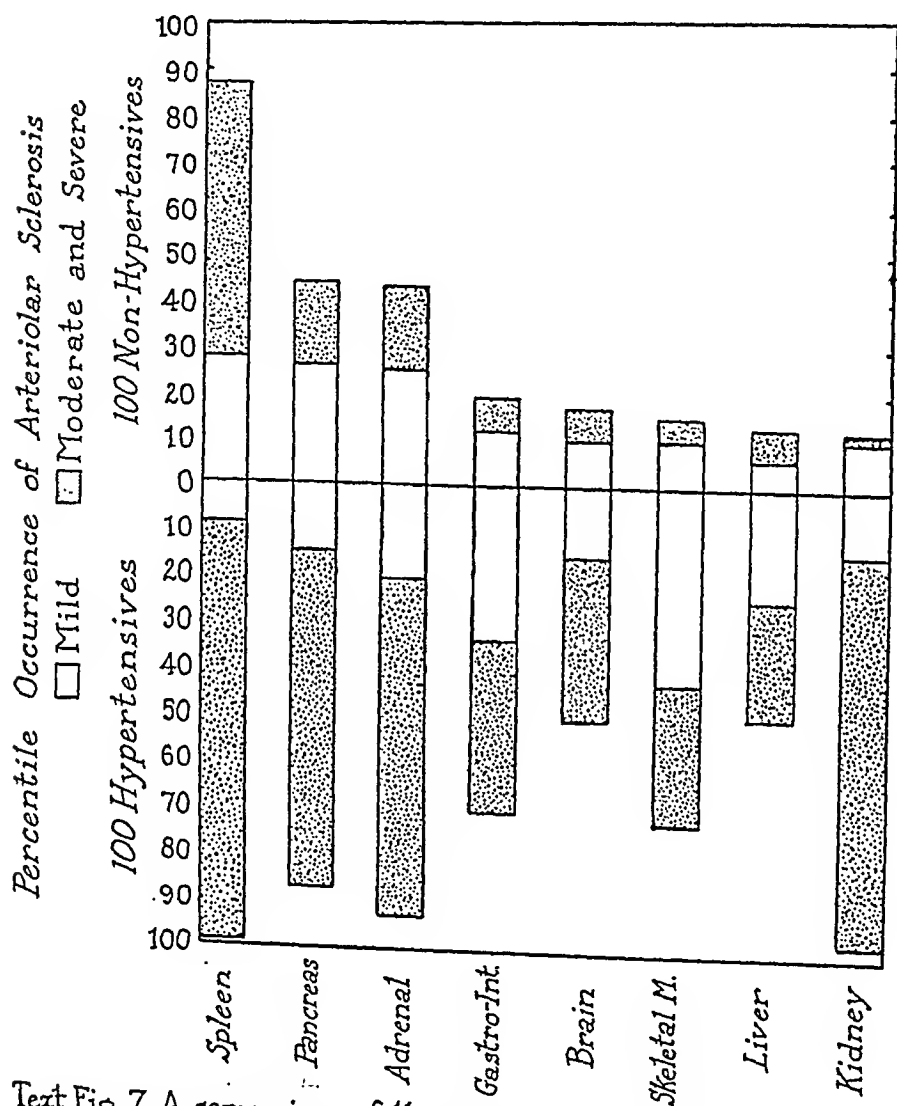
The microscopic changes in this type of arteriolar disease were similar to those occurring in other hollow muscular structures subjected to increased internal expansile pressure. It is not known whether vasoconstriction in hypertension is segmental or general, but if it is segmental it can be understood how dilatation might take place proximal to contracted segments or, as suggested by Volhard, may antedate the distal arteriolar spasm which results in the abnormal peripheral resistance. If medial disease is in some circumstances primary, it is conceivable that wall stretching might occur even with normal blood pressure and medial hypertrophy result. Although no conclusive evidence has been offered in support of the view that this medial change is secondary to stretching, such an explanation appears tenable.

In summary it may be said that the two most prominent and frequently occurring types of arteriolar disease, intimal hyalinization and intimal hyperplasia, appear to be primary pathological processes. The former is an aging phenomenon and the latter appears to be either an accelerated type of the former or an independent, non-exudative, productive arteriolitis. Medial hypertrophy appears on anatomical grounds to be a secondary phenomenon, probably related to stretching of vessels by the intravascular bursting tension. The medial degeneration may be in part primary, leading to smooth muscle stretching in non-hypertensive as well as in hypertensive individuals. Severe forms of arteriolar medial disease were seen only in persons with chronic hypertension.

#### CORRELATION OF ARTERIOLAR SCLEROSIS AND CHRONIC HYPERTENSION

In the foregoing review of the histological types, relative severity and distribution of arteriolar sclerosis in hypertensive and non-hypertensive individuals, two very pertinent facts have emerged. One is the high degree of correlation between renal arteriolar sclerosis and hypertension, and the other is the extreme rarity of more than a very mild degree of renal arteriolar sclerosis in non-hypertensive individuals (Text-Fig. 7). It should be recalled that the tissues from these 200 cases were examined objectively. Renal arteriolar sclerosis was recorded as being present in 109 persons. In 97 of these 109 cases of nephrosclerosis an

examination of the clinical records, corroborated by heart weights, revealed chronic hypertension to have been present. This permits the conclusion that at the time of death almost every chronic



Text Fig. 7. A comparison of the occurrence of arteriolar sclerosis in the various organs and tissues of 100 hypertensive and 100 non-hypertensive individuals.

hypertensive (97 per cent) has renal arteriolar sclerosis in some degree, that few non-hypertensives (12 per cent) have any renal arteriolar sclerosis, and that in only 2 per cent of the non-hypertensives is there more than mild arteriolar nephrosclerosis (see Text-Fig. 7). No comparable correlation could be found in the

case of any other organ or tissue. Although in hypertensives the arterioles of the spleen were frequently as sclerotic as those of the kidney, they were also found to be diseased in 86 per cent of the non-hypertensives. With the exception of the kidney, correlation of arteriolar sclerosis and hypertension is either not constant enough to indicate a significant relation or the organ or tissue shows arteriolar sclerosis so frequently in non-hypertensive persons that the significance of the positive correlation is destroyed.

Inquiry should next be directed at the causal relation of renal arteriolar sclerosis to hypertension. The two obvious possibilities are: (1) that hypertension causes the sclerosis of renal arterioles; and (2) that renal arteriolar sclerosis causes the hypertension.

The first explanation would attribute to the renal arterioles an extremely high degree of selective vulnerability to high blood pressure, since they were affected in almost every case of hypertension but were rarely diseased in any other circumstances. At the same time it would be necessary to attribute to the renal arterioles a high degree of resistance to every injury except the mechanical damage incident to elevated blood pressure. Arterioles in many parts of the body were found to be severely diseased in hypertensives, but with the exception of the kidney the same relative susceptibility of tissues to arteriolar sclerosis was seen in hypertensive and non-hypertensive individuals.

The second possible explanation, namely, that renal arteriolar sclerosis causes hypertension, is far more tenable. Arteriolar sclerosis occurs as a primary pathological change. It is entirely reasonable to assume that this primary vascular disease may affect the renal arterioles as well as those in the spleen, pancreas, adrenals and other tissues. When the renal arterioles become sclerotic, hypertension is almost invariably present. This conclusion is based on objective evidence in human postmortem material and is of especial significance because of the close correlation it establishes between essential hypertension in man and the experimental production of chronic hypertension in dogs by Goldblatt and collaborators. Goldblatt's original observations, subsequently confirmed by Page, Elaut, Wood and Cash, Collins, Harrison, Blalock and Mason, and Prinzmetal and Friedman, that reduction in blood flow through the main renal arteries of dogs by the use of clamps invariably led to chronic hypertension, is in entire accord

with the pathological anatomical findings in chronic hypertension in man. The renal arteriolar sclerosis in man appears to have the same functional effect as the silver clamp around the main renal arteries in dogs.

The fact that renal arteriolar sclerosis does in some instances occur without an associated hypertension in no way detracts from the major hypothesis. It is obvious that the reduction of blood flow through the kidney is not the entire mechanism involved in the production of chronic hypertension. Some humoral or reflex influence must lead to the generalized spasm of arterioles which makes hypertension possible. Even though the renal vascular disease with resulting reduction of blood flow through the kidneys is present, it is conceivable that other conditions are not favorable for the development of hypertension. A certain degree of myocardial competence and a sustained resistance to blood flow through the peripheral vessels is obviously necessary for the maintenance of high blood pressure. It is possible that as a result of natural causes or surgical intervention the functional response (hypertension) to reduced blood flow through the kidneys (arterial or arteriolar nephrosclerosis, chronic diffuse glomerulonephritis, congenital polycystic renal disease, and so on) may in some instances be inhibited. Furthermore, it is not possible to translate accurately structural change into functional effect. What appears morphologically to be mild arteriolar disease may in reality account for considerable reduction in renal blood flow, and the converse is equally true.

In this consideration of the correlation of chronic hypertension and the distribution of arteriolar sclerosis, attention must be paid to the 3 cases of chronic hypertension in which there was no significant degree of renal arteriolar sclerosis (see Text-Fig. 7).

The 1st case was a female, 54 years of age, who died of cardiac tamponade following the rupture of an aortic aneurysm. Blood pressure determinations prior to her terminal illness varied about the figure of 220/120. The heart weight was 450 gm. and there was severe renal arteriosclerosis.

The 2nd case was that of a female, 75 years of age, who died of pulmonary embolism following a period of mild cardiac decompensation. Her mean blood pressure during the period of observation was 160/90 and the heart weight was 680 gm. with-

out significant concomitant heart disease. At autopsy severe renal arteriosclerosis was observed and there were obstructing annular plaques around the aortic ostia of the renal arteries.

The 3rd case was a male, 60 years of age, who died of cardiac rupture through a myocardial infarct. Preceding the terminal infarct, blood pressure of 190/110 was observed. The heart weighed 500 gm. and there was a severe renal arteriosclerosis, with scattered coarse scars throughout both kidneys.

All 3 had renal arteriosclerosis of sufficient severity to constitute an independent postmortem diagnosis. If it be assumed that these were undoubted cases of chronic hypertension, their hypertension is susceptible to the same explanation as might be offered in the case of renal arteriolar sclerosis. The vascular changes are consistent with, although not definitely confirmatory of, reduced blood flow through the kidneys.

### TYPES OF CHRONIC HYPERTENSION

In these 100 cases of chronic hypertension there were neither clinical nor pathological criteria for establishing definite subgroups. Certainly there were some cases in which the disease ran a more acute course than in others, and such disease was found more frequently in persons under 45 years of age and usually terminated in death from renal insufficiency. This vaguely defined group has been spoken of as "malignant hypertension" in contrast to "benign hypertension" which affected an older group, progressed more slowly and characteristically terminated in heart failure or cerebral hemorrhage if not interrupted prematurely by accident or unrelated illness.

In reviewing the clinical data it appeared to be much easier to identify cases of chronic hypertension as malignant or benign in retrospect than it was to prognosticate the course of the disease from its beginning. There appeared to be no way of predicting the speed at which the disease would progress except on the basis of the rate of the cardiac or renal deterioration that had already occurred. Certainly the height and fixation of the blood pressure provided no constant index of the rapidity with which the disease would progress, nor did observation of the severity of vascular changes by ophthalmoscopic examination, except as they indicated the degree to which the disease had already progressed. Measure-

ments of a large number of arterioles in biopsy of pectoral muscle did not make it possible even to identify the presence of hypertension in more than half of the cases in which high blood pressure was present for a long time and provided no useful information in distinguishing between the "benign" and "malignant" form of the disease.

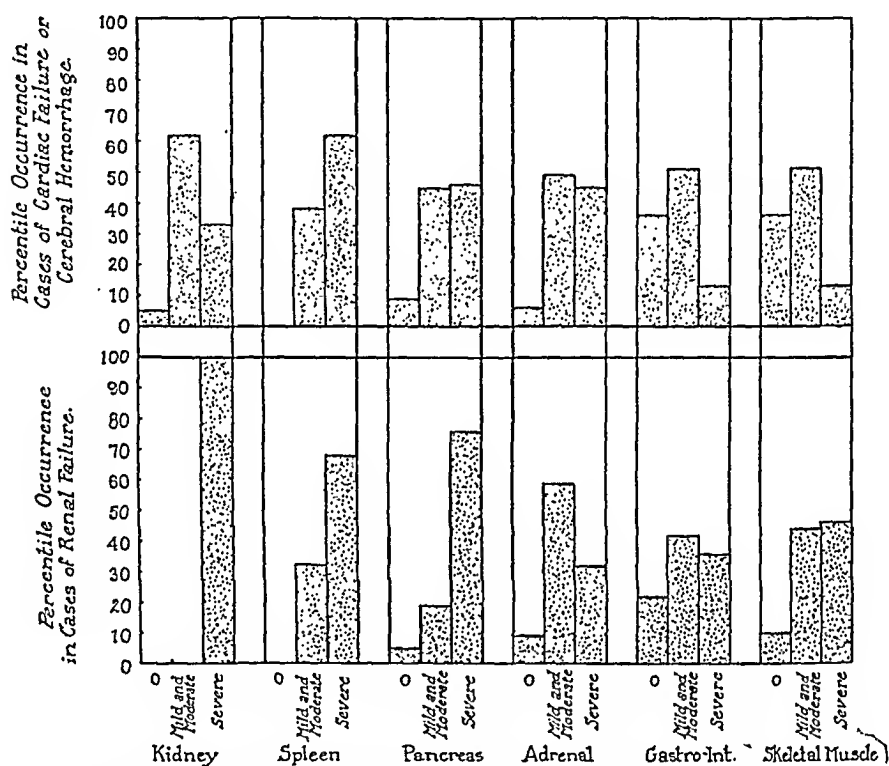
Certain group differences in the pathological anatomy of the so-called "benign" and "malignant" hypertension were apparent. The essential difference appeared to lie in the rapidity with which the vascular disease progressed in the kidney. If the arteriolar nephrosclerosis progressed with sufficient rapidity, renal insufficiency developed before the duration of the hypertension was sufficient to determine death from heart failure or cerebral hemorrhage. However, a number of examples were found of a rapidly progressing renal vascular disease in which heart failure or cerebral hemorrhage interrupted what might be properly regarded as the natural termination of the disease by renal failure. As a rule such cases were of older persons with severe coronary or cerebral arterial sclerosis.

In the material on which this investigation was based, in all the cases of so-called "malignant" hypertension severe generalized arteriolar nephrosclerosis was present. Not only was there occlusive intimal hyalinization of the afferent arterioles, but the small arteries from which they were derived were the seat of obliterating endothelial proliferation. A wide variety of acute degenerative and inflammatory changes was observed to occur in vessels already chronically diseased. These changes have been discussed in detail by Fahr, Klemperer and Otani, and Schurmann and MacMahon. An examination of Text-Fig. 8 suggests that on anatomical grounds this type of hypertension does not differ qualitatively from "benign" hypertension but is an accelerated and more severe type of vascular disease than is seen in the benign form. This conclusion is in essential agreement with the opinion of Herxheimer and Löhlein. This was especially true in the kidney, for in 100 per cent of the cases with death from renal failure, the vascular disease had been graded as severe.\* In every organ and

\* This does not imply that severe vascular changes are regularly seen in all cases of death from renal failure. In the 100 cases of chronic hypertension on which this study was based, all instances of death from renal insufficiency were by chance in cases of severe arteriolar nephrosclerosis.



tissue examined the arteriolar disease occurred with greater frequency and severity in the "malignant" than in the "benign" types of hypertension. Exclusive of the acute secondary arteriolar lesions, there was no type of vascular change in any tissue or organ that provided absolute grounds for distinguishing between malignant and benign hypertension. Although 100 per cent of the kidneys in cases of malignant hypertension showed severe



Text Fig. 8. A comparison of the occurrence, distribution and severity of arteriolar sclerosis in chronic hypertensives dying of renal failure and those dying of cardiac failure or cerebral hemorrhage.

arteriolar disease, equally severe renal arteriolar sclerosis was observed in 23 per cent of the cases of benign hypertension. Although 41 per cent of the cases of malignant hypertension had severe arteriolar lesions in skeletal muscles, 10 per cent of the benign hypertensives had equally severe arteriolar lesions in the skeletal muscles.

In both forms of hypertension the vascular disease was characteristically generalized and was represented by the same histological types of arteriolar lesions which were more severe and as a rule more widely distributed in individuals dying of renal

TABLE IX

*The Number of Patent Renal Glomeruli in Various Types of Chronic Hypertension \**

Death due to renal failure						Death due to cardiac failure or cerebral hemorrhage				
Age	Sex	Heart weight	Renal arteriolar sclerosis	Total No. of patent glomeruli		Age	Sex	Heart weight	Renal arteriolar sclerosis	Total No. of patent glomeruli
<i>yrs.</i> 55	F	<i>gm.</i> Enlarged	Severe	<i>thousands</i> 101		<i>yrs.</i> 67	M	<i>gm.</i> 780	Severe	<i>thousands</i> 378
27	F	450	Severe	169		64	M	500	Severe	452
58	M	460	Severe	240		38	F	510	Moderate	490
48	F	Enlarged	Severe	243		70	M	550	Severe	645
35	M	650	Severe	254		46	M	875	Moderate	703
41	M	470	Severe	269		50	M	650	Moderate	749
49	F	430	Severe	386		62	M	500	Moderate	834
44	M	600	Severe	422		41	F	830	Severe	908
49	F	500	Severe	412		70	M	700	Mild	908
40	F	450	Severe	459		57	F	600	Moderate	972
58	M	740	Severe	510		52	M	600	Moderate	988
40	F	540	Severe	654		40	M	600	Mild	980

\* The authors wish to express their appreciation to Dr. J. M. Hayman, Jr., for the glomerular counts.

insufficiency than in those dying of heart failure or cerebral hemorrhage.

Quantitative estimations of the degree of renal destruction by means of glomerular counts corroborated the impression gained from the histological examination and from the clinical course of the disease. Glomerular counts by Vimtrup's method were made on the kidneys of 24 chronic hypertensives (Table IX).

These 24 were comprised of two groups of 12 cases each. The mean age of the first group at the time of death was 45 years and of the second group, 55 years. Hypertension was generally of shorter duration in the first group in which death was the result of renal insufficiency. The second group had longer records of known chronic hypertension and death was due either to heart failure or cerebral hemorrhage. There were no significant differences in the degree of hypertension in the two groups. Cardiac hypertrophy was more pronounced in the group dying of heart failure or cerebral hemorrhage than in the group dying of renal insufficiency.

There was a striking difference in the numbers of patent glomeruli per kidney in the two groups. It has been shown by Moritz and Hayman that 1,250,000 is the approximate number of patent glomeruli in the normal kidney. In most of the individuals dead of renal failure there was a pronounced reduction in the number of injectible glomeruli, as compared with those dead of heart failure or cerebral hemorrhage. With full regard for the fact that a patent glomerulus is not necessarily a normal one, it is apparent that although the degree and duration of hypertension seem to be independent of the actual amount of renal destruction, death from renal insufficiency is associated with a measurable increase in the amount of glomerular destruction.

## CONCLUSIONS

### *General Characteristics of Essential Hypertension*

The mortality and probably the morbidity of essential hypertension was greater in blacks than in whites and the mean age at the time of death was lower in blacks than in whites.

The proportion of cases of essential hypertension with death

from renal insufficiency, heart failure and cerebral hemorrhage was the same in blacks as in whites.

There were no differences between males and females in mortality, cause of death or age at time of death from chronic hypertension.

The mean age at the time of death was lower in individuals dying of renal failure than in those dying of heart failure or cerebral hemorrhage.

In almost half of all cases of essential hypertension with death from heart failure, postmortem examination disclosed occlusive coronary disease.

### *Histological Types of Chronic Arterior Disease*

The three principal histological types of chronic arterior disease included: (1) intimal hyalinization; (2) medial hypertrophy and degeneration; and (3) intimal proliferation.

All three types, separately and in combination, were found in non-hypertensives as well as in hypertensives. Although no direct information was available as to the etiology of the three types there was indirect evidence in support of the view:

1. That intimal hyalinization is the arterior counterpart of simple arteriosclerosis, is essentially a degenerative change, and becomes more widespread and severe with advancing age.

2. That medial hypertrophy and degeneration are changes resembling those following distention of any hollow muscular structure, and although medial degeneration may be primary in some instances, yet the medial hypertrophy is probably secondary to stretching and was seen with greater frequency and severity in hypertensives than in non-hypertensives.

3. That endothelial hyperplasia with increase in elastic tissue and secondary degenerative changes was seen: commonly in association with inflammation where it was properly called endarteritis obliterans; commonly as an adaptive involutional change in vessels whose capillary beds have been reduced by parenchymatous atrophy; and commonly in cases of hypertension where it may have represented a primary vascular inflammation or an accelerated form of arterior sclerosis.

*Measurements of Arterioles (Medial Hypertrophy)*

Neither the mean wall to lumen ratio, the mean external nor the mean internal diameter of large samples of arterioles from the skeletal muscle were useful in distinguishing between hypertensive and non-hypertensive individuals. Relative arteriolar wall thickening was a group characteristic for the hypertensives and, so far as histological preparations of tissues were concerned, the thickening resulted in an increase in external diameter rather than a decrease in the lumen diameter. The most useful numerical value in distinguishing hypertensives from non-hypertensives was the mean external diameter of arterioles within a fixed size range. It was possible to identify 48 per cent of the hypertensives by measurements that were in excess of those seen in any non-hypertensives, and it was possible to identify 16 per cent of the control cases by measurements lower than were observed in any of the hypertensives. Measurements of arterioles were not useful in distinguishing between various types of essential hypertension.

Neither the physiological state of the vascular system at the time the specimens were obtained for microscopic examination nor variations in the methods used in preparing tissues for microscopic examination led to changes in arteriolar dimensions that were greater than those resulting from anatomical variations or errors in mensuration under controlled conditions.

*Correlation of Arteriolar Disease and Chronic Hypertension*

The objective examination of arterioles in all parts of the body of 100 control cases and 100 cases of chronic hypertension disclosed only one situation in which the presence of arteriolar sclerosis was almost invariably associated with hypertension and where the absence of arteriolar sclerosis almost invariably betokened an absence of high blood pressure. This was in the kidneys. Renal arteriolar sclerosis was present in 109 of the 200 cases studied, and 97 of these 109 proved to be cases of chronic hypertension. No comparable correlation could be found in any other organ or tissue.

It was felt that these facts, together with the information gained from a study of the histological characteristics of arteriolar disease in hypertensive and non-hypertensive individuals, supported

the conclusion that renal arteriolar sclerosis is the most common cause of chronic hypertension. This conclusion is in accord with the recent demonstration by Goldblatt that chronic hypertension is regularly produced in dogs and monkeys by reducing the blood flow through the kidneys (renal ischemia). The effect of the renal arteriolar sclerosis in human hypertension appears to be the functional analogue of the renal arterial clamp in experimental hypertension. In both instances hypertension appears to be produced by reduction in renal blood flow which does not necessarily lead to a sufficient degree of ischemia to impair renal function measurably.

It is concluded that the only significant site of arteriolar sclerosis so far as the causation of hypertension is concerned is the kidney.

### *Types of Chronic Hypertension*

The material on which this investigation was based did not include cases in which the hypertension could be attributed to chronic nephritis, congenital polycystic renal disease, urinary obstruction, obesity, hyperthyroidism, aortic insufficiency, aortic coarctation, pituitary or adrenal tumors, or arteriovenous aneurysm. Such causes of hypertension were not excluded deliberately but were simply not encountered in the selection of cases by the criteria outlined in the early part of this report. A survey of the rate of progress of the disease, the mean age at the time of death and the cause of death disclosed two groups which were best separated by the manner in which the disease terminated. One group died of renal insufficiency and for several reasons was most adequately described as "malignant" hypertension in contrast with a group that died of heart failure or cerebral hemorrhage which was designated as "benign" hypertension. The application of the terms "malignant" and "benign" denoted group characteristics applicable in retrospect rather than constituting useful terms in predicting the course of the disease in its early stages. The factor which determines that some cases of essential hypertension will run a more rapid course with early death from renal insufficiency does not appear to be the degree of the hypertension but rather the rate of renal destruction incident to the progressive character of the renal vascular disease. Although no correlation could be established between varying degrees of renal vascular disease and vary-

ing degrees of blood pressure elevation, there did appear to be a correlation between the amount of renal atrophy, as indicated by glomerular counts and the mode of death.

Note: The authors wish to express their indebtedness to Miss Theodora Bergsland for the colored illustrations.

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## DESCRIPTION OF PLATES

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### PLATE 98

#### Intimal Hyalinization

FIG. 1. Arteriole showing plaque of intimal hyalin.

a = Hematoxylin-eosin stain.

b = Combination of van Gieson's and Weigert's elastic methods on same section.  $\times 500$ .

FIG. 2. Annular form of intimal hyalinization showing beginning degeneration of internal elastic lamella incorporated within the hyalin.

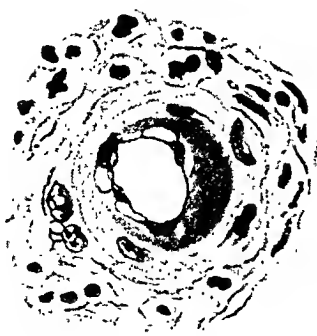
a = Hematoxylin-eosin stain.

b = Combination of van Gieson's and Weigert's elastic methods on same section.  $\times 500$ .

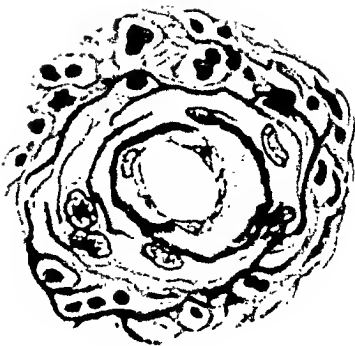
FIG. 3. Intimal hyalinization in a dilated, thin-walled vessel.

a = Van Gieson's method.

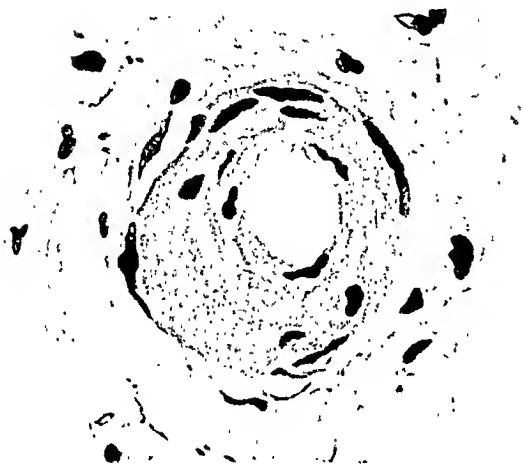
b = Weigert's elastic method on same section.  $\times 500$ .



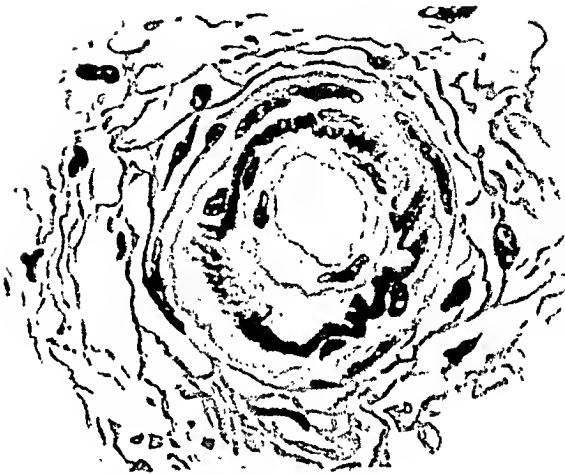
1a



1b



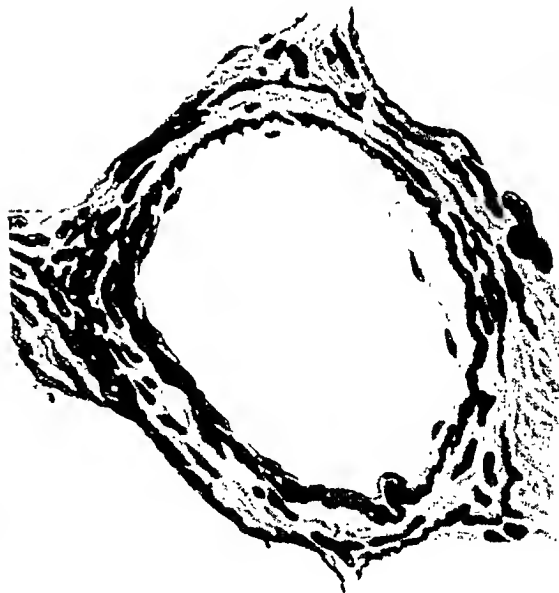
2a



2b



3a



3b

PLATE 99

Medial Hypertrophy

FIG. 4. Normal arteriole showing increased wall thickness as the result of pronounced postmortem contraction.

a = Hematoxylin-eosin stain.

b = Combination of van Gieson's and Weigert's elastic methods on same section.  $\times 500$ .

FIG. 5. Hypertrophy of media.

a = Hematoxylin-eosin stain.

b = Combination of van Gieson's and Weigert's elastic methods on same section.  $\times 500$ .

FIG. 6. Collagenous degeneration and medial hypertrophy.

a = Hematoxylin-eosin stain.

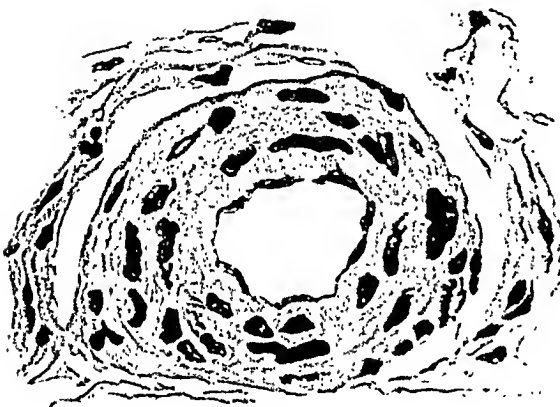
b = Combination of van Gieson's and Weigert's elastic methods on same section.  $\times 500$ .



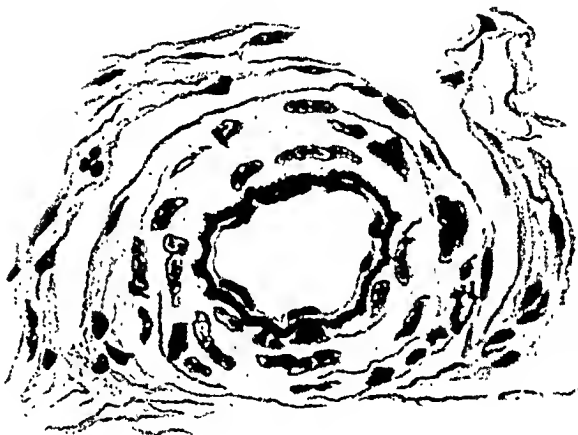
4a



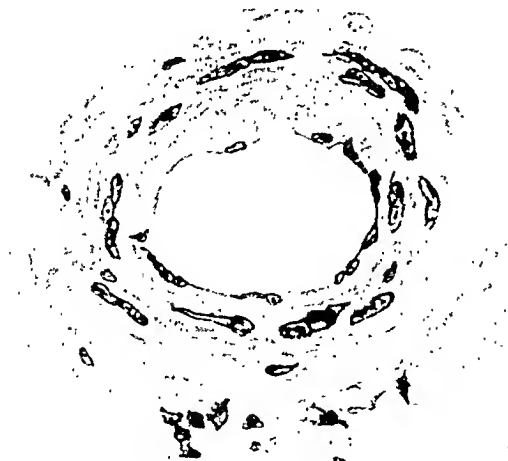
4b



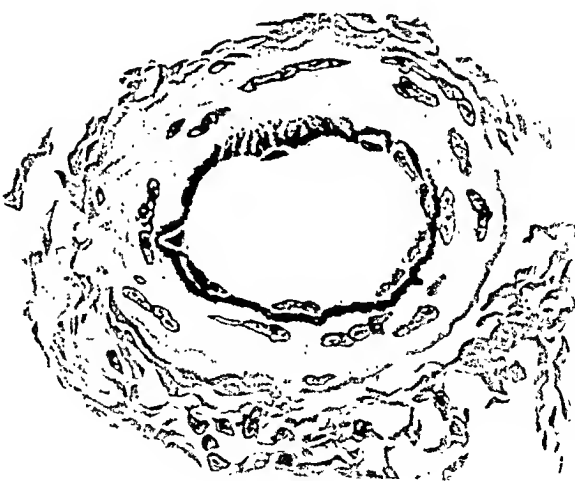
5a



5b



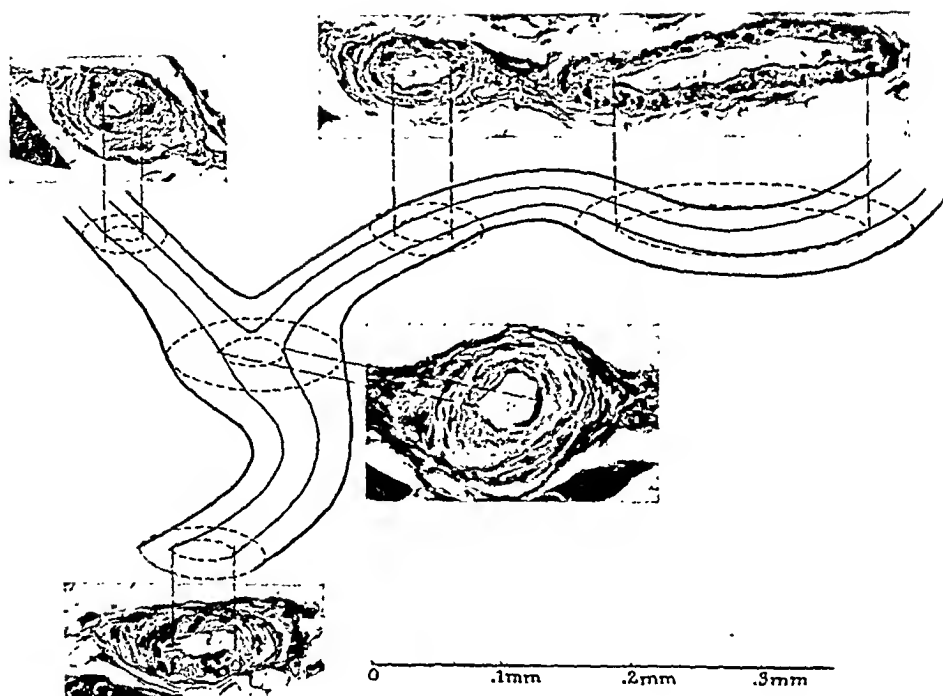
6a



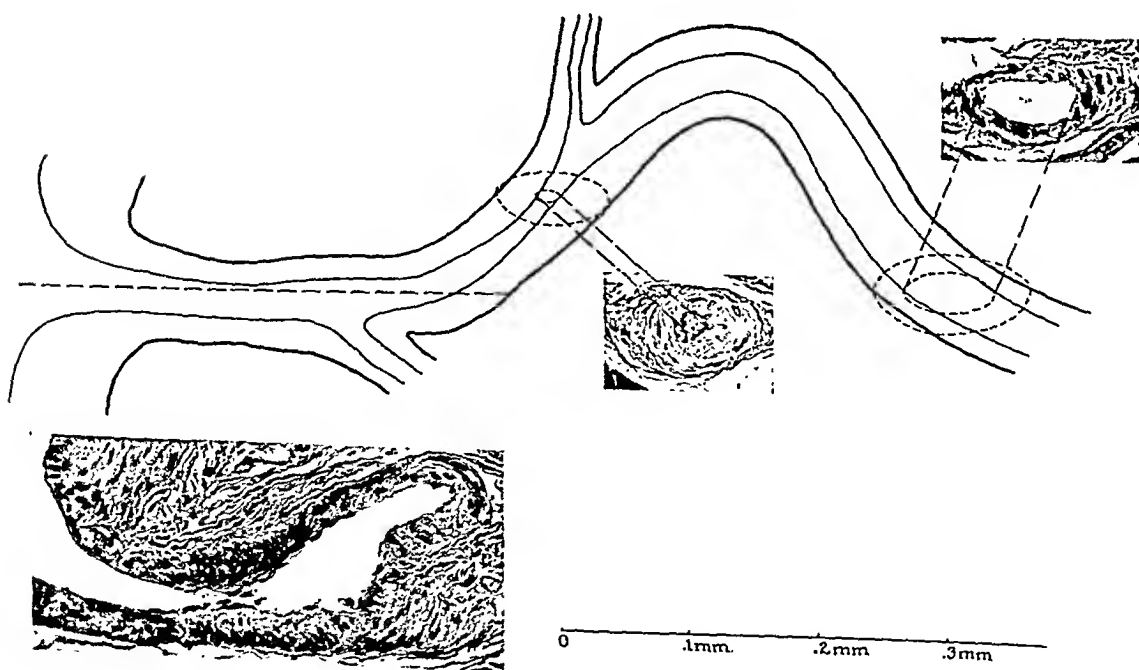
6b

PLATE 100

- FIG. 7. Reconstruction of an arteriole from the pectoral muscle of an individual with chronic hypertension showing the non-uniform character of the vascular disease in different segments of the same vessel.
- FIG. 8. Reconstruction of an arteriole from the pectoral muscle of an individual with chronic hypertension showing the non-uniform character of the vascular disease in different segments of the same vessel.



7



8

PLATE 101

Endothelial Hyperplasia

FIG. 9. Endothelial hyperplasia within an intact internal elastic lamella with pronounced intimal thickening and reduction in lumen caliber.

a = Hematoxylin-eosin stain.

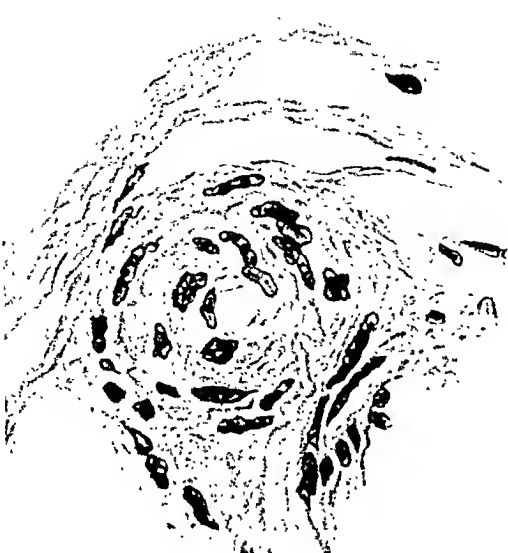
b = Combination of van Gieson's and Weigert's elastic methods on same section.  $\times 500$ .

FIG. 10. Endothelial hyperplasia associated with reduplication of internal elastic lamella and degeneration of old and new elastic tissue.

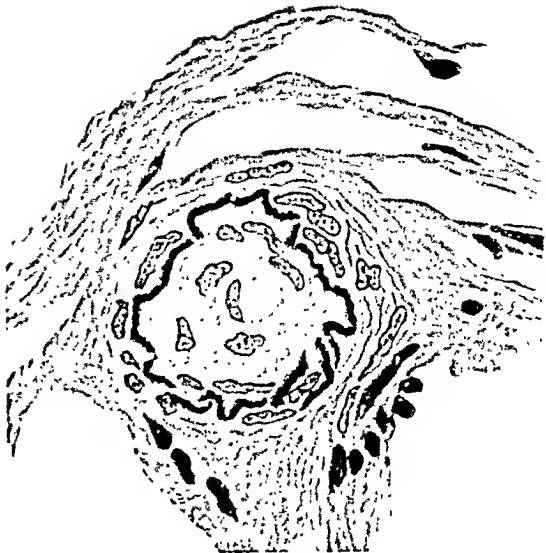
a = Hematoxylin-eosin stain.

b = Van Gieson's method.

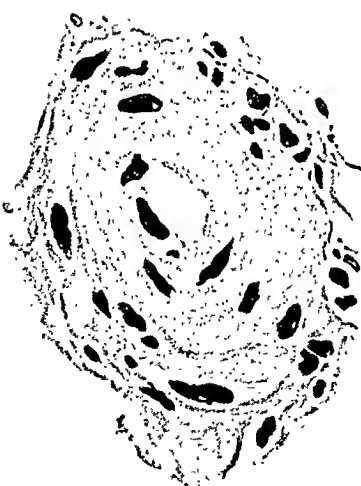
c = Weigert's elastic method.  $\times 500$ .



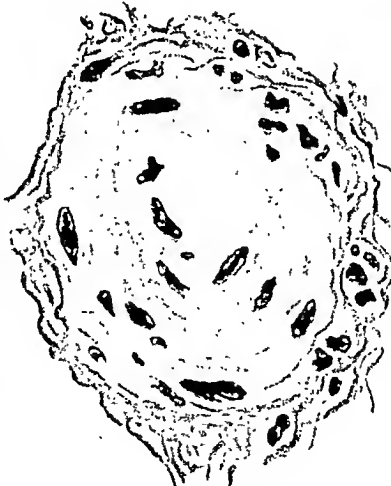
9a



9b



10a



10b



10c

Moritz and Oldt

Arteriolar Sclerosis





# INTRANUCLEAR INCLUSION BODIES IN THE TISSUE REACTIONS PRODUCED BY INJECTIONS OF CERTAIN FOREIGN SUBSTANCES \*

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In studies on prevention of experimental equine encephalomyelitis in guinea pigs by means of virus adsorbed on aluminum hydroxide Cox and Olitsky <sup>1, 2</sup> observed in the phagocytic mononuclear and giant cells of the induced subcutaneous nodules intranuclear inclusion bodies characteristic of encephalomyelitis virus infection. When the chemical alone, free from virus, was introduced under the skin of guinea pigs, similar inclusions were seen in the resulting foreign body reaction. This finding gave a point of departure for the present investigation in which an attempt was made to study further the nature and significance of the nuclear changes brought about through the action of non-virus material.

Intranuclear inclusion bodies are found as characteristic changes in the lesions of many virus diseases.<sup>3, 4</sup> In addition they have often been observed in tissues in which no search was made for the presence of virus although infection with virus was strongly suspected.<sup>5, 6</sup> Cowdry,<sup>3, 4, 7</sup> however, has stated that such structures are not necessarily the result of virus action and has held to the possibility that they may be produced experimentally by other means. A number of investigators, using non-infective materials, have produced bodies resembling the inclusions. It is noteworthy that in none of these procedures have attempts been made to eliminate the presence of virus occurring spontaneously in the animal tissues. (Summaries of and references to such reports are given in several articles.<sup>4, 8-12</sup>)

A wide variety of methods and materials was used in these recorded instances. Bodies which investigators or their later commentators believed to resemble virus inclusions were found in tissues moistened with ammonium chloride followed by applica-

\* Received for publication May 11, 1937.

tions of direct electric current; in nerve cells repeatedly stimulated by electric current; in ganglionic cells soaked in hypertonic sodium and calcium chlorides; in rabbit corneas injected with bacterial toxins, and in pad tissues injured by heat or acid. Among other materials used were: hypertonic solutions of glucose, sodium chloride, and bicarbonate; bismuth, lead, arsenic, morphine, strychnine, salyrgan, exotoxins, and so on, given to animals by various routes of inoculation.

In certain of these reported cases the nuclear changes might be regarded as not conforming with inclusions typical of virus infection. In some the entire nucleus was reported as shrunken<sup>13</sup>; in others, suspected bodies could well have been degenerated nucleoli which stained pink instead of blue<sup>12</sup>; in still others the basophilic chromatin was clumped about the nucleolus,<sup>14</sup> but in the remainder some resemblance to virus inclusions was apparent; for example, certain ones produced by Lee<sup>15</sup> with salyrgan were, in Cowdry's opinion,<sup>4</sup> indistinguishable from structures produced by viruses. As already mentioned, however, in none of these cases have attempts been made to exclude the possibility of a preexisting or concomitant virus as the causal factor in the production of the structures.

The following criteria are generally considered as the characteristic features of true virus inclusions. Their morphology must, of course, be based on studies of structures found only in virus-containing tissues; that of bodies which have no proved relationship with any virus is not applicable. By many investigations on undoubted virus inclusions it has evolved that no single characteristic feature is invariably found but that three particular properties are of outstanding importance, namely, (1) acidophilic staining, (2) halo formation, and (3) margination of chromatin with or without displacement of nucleoli. Cowdry<sup>7, 16</sup> has attempted to define the nuclear inclusions more precisely and to obtain clues of their chemical constitution by the use of histochemical reactions. Most noteworthy of these are the Macallum test for masked iron and the Feulgen reaction for thymonucleic acid; both give positive reactions with nuclear chromatin and negative results with virus inclusions.

Nuclear inclusions have also been classified in two types by Cowdry. Type A includes generally bodies in nuclei in which all

of their basophilic chromatin is ultimately margined; such are found in herpes, yellow fever, virus III disease, pseudorabies and others. In Type B the basophilic chromatin fails to marginate and the acidophilic bodies appear in various parts of the nucleus; they occur in poliomyelitis, Borna's disease, equine encephalomyelitis, and others. Both types may, however, occur in the same disease, as shown by Cowdry,<sup>4</sup> Sabin and Hurst,<sup>17</sup> and by our own observations.

The general characteristics which are commonly ascribed to proved virus inclusions have been mentioned but it is known that variations occur. Most specific intranuclear inclusions are acidophilic but some have been described<sup>17-19</sup> that are either partially or completely basophilic. Presence of halos depends on the particular virus and the stage of infection. In herpes<sup>20</sup> halos appear only in later stages. Margination occurs irregularly; Cowdry, Lucas and Fox<sup>21</sup> have noted it in normal cells and we have observed it in nuclei without inclusions. The histochemical reactions have not been universally applied.

#### METHODS AND MATERIALS

Aluminum hydroxide was prepared essentially according to the directions for making Willstätter's Type C gel.<sup>22</sup> This was suspended in  $\frac{M}{30}$  phosphate buffer at pH 6.6. Another preparation was secured by adding equal parts of ammonium hydroxide (C. P., 28 per cent  $\text{NH}_3$ ) and a solution of 32 per cent aluminum sulphate with shaking at room temperature. The resulting precipitate was washed with distilled water until free of ammonia, using the centrifuge for sedimentation. A third form of aluminum consisted of alundum, an insoluble form of aluminum oxide commonly used as an abrasive in grinding virus-containing tissues. A weighed amount was thoroughly ground in a mortar with distilled water until a fine suspension resulted. Ferric hydroxide was obtained by adding ammonium hydroxide to a clear solution of ferric chloride and washing in the same way as the Type C gel. Suspensions of barium sulphate, silver chloride and carbon were prepared by adding distilled water to the required amounts. Parowax (a paraffin of moderately low melting point) and 2 per cent nutrient

agar with Witte's peptone were also used. All preparations were autoclaved.

Materials were injected under the skin in guinea pigs and rabbits in amounts of 1 cc. in each site. The object was to produce palpable nodules that could be removed at various intervals of time. It was found that size and persistence of nodules depended mainly on concentration and composition of the material, the depth of injection, and the thickness of the skin. Those of aluminum hydroxide usually lasted 6 to 8 weeks. Ordinarily 7.5 to 10 mg. per cc. of aluminum oxide or of the other chemicals were used but concentrations as high as 20 to 60 mg. per cc. of ferric hydroxide, barium sulphate, or carbon were sometimes necessary to bring about persistent nodules. Paraffin was given undiluted. When the first injection did not result in the formation of nodules, the same sites were reinjected 2 or 3 days later with the same or a higher concentration of the same material. The time of removal of nodules was then counted from the date of second injection.

Nodules were excised \* aseptically at various intervals. The skin was closed by clips and small dressings of gauze with collodion were applied. In this way 4 or 5 nodules could be removed from a single animal. The excised tissue was cut so that a piece of skin was retained and then fixed in Zenker's solution containing 5 per cent glacial acetic acid in preparation for ordinary stains to be mentioned, and in special fixatives for certain other stains, as shown in Table II.

#### TYPES OF TISSUE REACTIONS

During the first few days after subcutaneous inoculation of the mentioned foreign substances in guinea pigs (and in the case of aluminum compounds and of ferric hydroxide, in rabbits), the tissues at the site of injection responded with the usual type of inflammation — chiefly polymorphonuclear cell invasion, edema and congestion. After 4 or 5 days the individual reaction to each of the substances became manifest.

Table I summarizes the types of induced reactions. With the aluminum substances, the hydroxide C gel or ordinary form, or alundum, a foreign body nodule was brought about. The central

\* Operations on animals were done with the aid of ether anesthesia.

portion contained heterogeneous material of broken down cells, the injected chemical itself, débris, and a few polymorphonuclear

TABLE I

*Substances Used for Injection and Their Effects in Guinea Pigs*

Substance	Main features of tissue reaction 1 week after inoculation	Presence of inclusions
Aluminum hydroxide (Willstätter's Type C)	Phagocytic mononuclear cells and giant cells	++*
Ordinary aluminum hydroxide	Same	++
Alundum (Al <sub>2</sub> O <sub>3</sub> )	Same	++
Ferric hydroxide	Same	+
Barium sulphate	Same	—
Silver chloride	"Cold" abscess surrounded by newly formed connective tissue. No giant cells	—
Paraffin	Strands of fibroblasts surrounding and penetrating the paraffin. Few phagocytic mononuclear and giant cells	—
Carbon	Phagocytic mononuclear cells and giant cells	+
Agar	Same	—

\* ++ indicates that numerous inclusions occurred from about the 7th day after inoculation and then persisted throughout the duration of the lesion (for several weeks).

+ indicates that they occurred only temporarily and in small numbers.

and monocytic leukocytes. This was surrounded by a dense mass of phagocytic mononuclear and later of giant cells (Figs. 1 to 6).<sup>\*</sup> A connective tissue band of varying width was formed at the outer

<sup>\*</sup> With certain chemicals (aluminum, iron) the giant cells contained nuclei arranged peripherally; with others (barium, paraffin, and carbon), these cells exhibited nuclei that were scattered throughout the cytoplasm, and with agar both forms, especially the former, were produced. The foreign body was, however, engulfed by the cytoplasm of both types of giant cells. Supravital and Masson's stains were performed only on the aluminum material. Here the precise classification of cells comprising the nodular lesion could be made (with the assistance of Dr. A. L. Joyner) and revealed phagocytic mononuclear, stimulated phagocytic mononuclear, epithelioid, and Langhans' giant cells (those with peripherally arranged nuclei).

margin. The phagocytic mononuclear and giant cells progressively invaded the central material from its periphery and engulfed most of it. The process continued until all miscellaneous contents of the center were removed, ultimately to end in healing by scar tissue formation. The lesions induced by ferric hydroxide were similar. The iron in stained or unstained nodules could be easily identified by its pronounced brown color both in cytoplasm and in the central area (Fig. 7). Barium sulphate after inoculation gave rise to nodular formation, the principal cells in which were phagocytic mononuclear and giant cells. The chemical could be seen both in the cytoplasm of these cells and in the core of the lesion as colorless, ice-like crystals. Silver chloride produced a different kind of lesion — a "cold" abscess of polymorphonuclear cells surrounded by a band of newly formed connective tissue cells, terminating finally in fibrosis. No giant cells were present. With paraffin were found strands of fibroblasts surrounding and penetrating the substance which was not seen in sections since solvents used in preparation had removed it. The lesion also contained a few phagocytic mononuclear and giant cells. Carbon induced localized areas of these cells surrounded by a connective tissue membrane. The black carbon particles were found to be packed into the cytoplasm of both forms of cells. Agar brought about nodules<sup>23</sup> which consisted of these cells with a thin layer of fibroblastic tissue enclosing the lesion. In earlier stages (to 7 days) agar as thin acidophilic strands was noted in the center and as amorphous material in the cytoplasm; later, the agar disappeared except for small, indefinite cytoplasmic masses.

To summarize, there were found in the lesions induced by agar and the chemical substances, except silver chloride, a preponderance of phagocytic mononuclear and giant cells. In those cells in which the cytoplasm visibly engulfed the foreign body, no trace of their presence could be seen in the nucleus (Figs. 3 to 7). The apparent failure to penetrate the nucleus when the cytoplasm was packed to the bursting point by the injected material should be stressed since a problem on the source of any intranuclear inclusions must first take into consideration the possibility of intranuclear entry of particles of the foreign body itself. This negative finding, taken together with others to be described, points to the fact that such invasion did not occur.

## INCLUSION BODIES

*Location and Morphology:* Inclusions were present only in the nuclei of the phagocytic mononuclear and giant cells which were produced by aluminum compounds, ferric hydroxide, and carbon, and not in the nuclei in the lesions induced by the other materials. In the aluminum nodules they appeared in small numbers about 7 days after injection, that is, a short time after the phagocytic mononuclear cells were apparent in large numbers. They increased progressively until at about the 24th day there were often 10 to 12 per oil immersion field. Nodules from different animals, however, showed some variation in numbers. How long they persisted depended on the duration of the nodule. Inclusions were first seen with ferric hydroxide on about the 18th day and then only in half the number of animals. At about the 24th day they were not demonstrable. However, when present they were quite numerous. With carbon the acidophilic intranuclear bodies were observed first on the 18th day after inoculation, then again on the 24th day. Thereafter the lesion, as formed by the described method, disappeared. In numbers the bodies were less than in the aluminum material and roughly equaled those found in the iron preparations.

Although the aluminum compounds and ferric hydroxide produced the same reaction in rabbits, yet the inclusions were extremely rare or absent in this animal. This was in contrast with guinea pigs in which they were invariably found after the first 7 days. As a corollary to the presence of inclusions in one species of animal and not in another, reference should be made to the work of Hurst<sup>18</sup> who could not demonstrate intranuclear inclusions in pigs infected with pseudorabies virus, although they were present in other animals, such as the rabbit, guinea pig, monkey and cow.

In stained preparations in the instances in which aluminum was injected, the contrast between the appearance of nuclei containing inclusions and those free from these bodies was striking. Non-inclusion-bearing nuclei were regularly basophilic. Such a nucleus consisted of a round or ovoid nuclear membrane within which were usually one or more nucleoli of various sizes and shapes. In addition, other more finely divided basophilic material was scat-



tered throughout. Minute amounts of acidophilic substance were also rarely seen but were not considered to be inclusions because of their small size, their lack of form, and their occurrence in otherwise normal cells. Amphinucleoli (basophilic nucleoli with acidophilic cores) were not ordinarily present. Basophilic chromatin was occasionally found collected on the nuclear membrane making it appear thicker and comparable to the margination of inclusion-bearing nuclei.

A constant characteristic of the inclusions was their property of acidophilic staining and this was used throughout as the most important criterion for their recognition (Figs. 3 to 7). No structure was thought to be an inclusion that did not stain with phloxine or some other "acid" dye and this made it necessary that differentiation be carried out properly during the staining procedure. Unless the nucleoli of the cells of adjacent tissues were definitely basophilic, the preparation was not considered suitable.

Most of the acidophilic bodies were approximately equal in size to average nucleoli. Others were larger, however, and some nearly filled the nucleus and could be seen without an oil immersion lens (Fig. 2). Great variation was observed with regard to form. Round, flat, whole-edged ones were common; some were angular, others fluffy. A number of them appeared to be composed of minute granules. Ordinarily they appeared less dense than nucleoli and more refractile (Fig. 2). As many as four were found in some nuclei, although one or two was the usual number.

In most instances margination of chromatin occurred, the nucleoli and other basophilic material being found collected peripherally on the nuclear membrane. Clear spaces or halos were usually to be seen around the inclusions. Nuclei containing the bodies at times were slightly larger than their fellows and appeared as a rule as clear sacs in which the ground substance could not be seen.

From their general appearance and also from their morphological characteristics it would seem that the inclusions produced by the substances mentioned are not distinguishable from those that occur in virus-containing tissues. Variations in form occur so that ones corresponding to either of Cowdry's types have been found in a single preparation. From the results of tests to be described immediately, additional similarity can be shown to exist between the chemically induced inclusions and those of virus diseases.

*Tinctorial and Histochemical Tests:* The purpose of these tests was to determine whether the intranuclear inclusions induced by the action of aluminum and iron compounds were (1) identical in their reaction to those brought about by viruses, (2) merely particles of the introduced foreign bodies, or (3) degenerated nucleoli.

Most of these tests were performed on nodules resulting from injection of aluminum compounds since these showed uniform formation of abundant characteristic intranuclear inclusions. In Table II will be found a summary of the reactions of the inclusion, the nuclear structures, and the aluminum material engulfed in the cytoplasm.

It will be noted that the inclusions evoked by means of chemicals maintain their acidophilic character with different stains, as is the case with those brought about by viruses. The only exception is iron hematoxylin. With the same stain, however, Sabin and Hurst<sup>17</sup> found the inclusions of B virus to be occasionally basophilic. Moreover, with most of the stains, color differences could be seen between inclusions and the aluminum hydroxide present in the center of the nodule or the cytoplasm of the cell. This suggested that the inclusions were not composed of aluminum hydroxide but did not exclude the possibility that within the nucleus it might have a different color because of combination or adsorption with organic substance there. However, as we have already stated, it was apparent that of all the chemical substances and agar injected, none was found by microscopic examination to have penetrated the nucleus.

The next series of tests concerned the possibility of identifying the inclusions with nuclear material. The Feulgen reaction is a test that has been widely used to detect the presence of thymonucleic acid which is a component of nuclear substance. Cowdry<sup>7, 16</sup> has found that the intranuclear inclusions of certain virus diseases do not give the reaction. It was applied to the inclusions under investigation following his method, except that in addition counterstains of eosin or orange G were used. Like those of virus diseases, these inclusions gave a negative reaction in contrast with the other parts of the nucleus and so presumably did not contain thymonucleic acid.

TABLE II

*Staining Reactions of Intranuclear Inclusions in Nodules Produced by Aluminum Hydroxide*

Stain, counterstain	Fixative	Inclusions	Nuclear membrane and nucleoli	Aluminum hydroxide in cytoplasm
Methylene blue, phloxine	Zenker's (5% acetic acid)	Red	Dark blue	Lavender or unstained
Methylene blue, eosin	Zenker's (5% acetic acid)	Pink or red	Dark blue	Lavender or unstained
Hematoxylin, eosin	Zenker's (5% acetic acid)	Dark red	Blue-black	Lavender or unstained
Hematoxylin, orange G	Zenker's (5% acetic acid)	Gray	Dark blue	Light yellow or unstained
Giemsa	95% alcohol	Dark red	Dark blue	Gray, laven- der, or un- stained
Methyl blue, eosin (modified Mann)	Zenker's (5% acetic acid)	Red	Blue	Lavender or unstained
Iron hematoxylin, orange G	Zenker's (5% acetic acid)	Black	Black	Yellow
Masson's connec- tive tissue stain	Helly's fluid	Dull brown or dark gray	Brown	Bright green
Feulgen, eosin	95% alcohol or sublimite alcohol	Red	Purple	Light pink or unstained
Feulgen, orange G*	95% alcohol	Orange	Purple	Light yellow or unstained
Masked iron, eosin	95% alcohol	Dull red	Blue-black	Lavender or unstained
Masked iron, orange G	95% alcohol	Yellowish gray	Blue-black	Light yellow or unstained

\* This counterstain was used as recommended by J. A. de Tomasi (*Stain Technology*, 1936, 11, 137).

Cowdry<sup>16</sup> has also found that the virus inclusions he studied reacted negatively to the Macallum test for masked iron. This is a test for iron in organic combination such as is present in the basophilic chromatin of normal nuclear material. The procedure was carried out essentially according to the directions of Nicholson<sup>24</sup> and Lee.<sup>25</sup> Sections were first allowed to stand in a solution of 4 per cent sulphuric acid in order to "unmask" the iron, that is, change it into inorganic form. This was followed by a yellow solution of pure crystalline hematoxylin \* which combined with this iron and gave a blue color. A counterstain of eosin or orange G was then applied. Again like those of virus diseases, these inclusions gave a negative reaction for masked iron.

Preliminary work with Zenker-fixed tissue on the application of the test for masked iron to nodules of ferric hydroxide showed that this substance itself did not react but retained a brownish yellow color similar to that in sections stained by ordinary methods. It was therefore considered unlikely that this method could give conclusive evidence as to whether ferric hydroxide had appeared within the nucleus modified in such a way as to stain as inclusions. However, such a possibility seemed remote in view of the fact that this iron compound showed such a distinctive color and apparently nowhere penetrated the nucleus.

The method of microincineration has also been applied to determine the mineral content of several virus inclusions.<sup>20, 26-28</sup> The procedure was employed in the present study essentially as described by Scott.<sup>28</sup> With the material studied, the results were disappointing since the nodules contained quantities of inorganic substance which left an abundant ash after the organic material was burned away. The ash left by the aluminum hydroxide covered the whole area in which inclusion-bearing nuclei were to be sought, thus obscuring the fields under observation.

*Other Tests:* Sodium alizarin monosulphonate added to salts of aluminum gives a red color; this reaction offers a delicate test for this element. Attempts were made to develop a histochemical test using this dye with the idea of detecting the presence of

\* When the preliminary treatment with acid was omitted from control sections the nuclei were not colored with this solution. 95 per cent alcohol was used for fixation because when such a control was used with sections of tissue fixed in Zenker's solution the nuclei gave a non-specific reaction.

aluminum in the inclusions. All such trials have ended in failure because the aluminum hydroxide in the center of the nodule or in the cytoplasm would itself never give a positive test. It is a very insoluble substance and any procedure designed to bring it into solution has a deleterious effect on the organic structures in the section. That aluminum was still present at the times nodules were removed was shown by gross chemical tests.

The method of supravital staining was tried on inclusion-bearing nodules. One could see the nucleolus eccentrically placed in the nucleus and suggestions of margination of nuclear material. Moreover, in some of such nuclei, bodies lighter in texture, more homogeneous, and resembling inclusions were seen. However, it could not be said with certainty that these were the same structures that were seen in fixed preparations.

From the results of these tests it would seem that the intranuclear inclusions evoked by the injection subcutaneously in guinea pigs of the aluminum and iron compounds have many resemblances to virus inclusions. The similarity exists not only in morphology and location but in the response of the bodies to the Feulgen and masked iron tests. Furthermore, the recorded reactions point to the fact that the inclusions under investigation are not merely degenerated nucleoli or artefacts due to technical procedures, and finally that they do not in themselves represent particles of injected chemicals.

### SEARCH FOR VIRUS

The presence of inclusions suggested the action of a virus (1) preexisting in the animals and activated by the injected materials; (2) "formed *de novo*" as questioned by Cowdry<sup>3, 7</sup> as a possible occurrence if intranuclear inclusions were produced by artificial means; (3) being a contaminant, perhaps one of the viruses studied in this laboratory, and (4) carried along with the materials used. The fourth can be eliminated by the fact that such substances were autoclaved. Because of the other three possibilities, evidence of a virus was sought. An outstanding example of where the finding of intranuclear inclusions led to the discovery of a virus is that afforded by the work of Cole and Kuttner<sup>20</sup> on the salivary gland virus of guinea pigs. This infective agent is ap-

parently present in most of the guinea pigs of certain stocks without giving rise to symptoms but is, nevertheless, associated with intranuclear inclusions. Such inclusion-containing material is, however, capable of transmission in series to young guinea pigs which then show the clinical disease with death.

*Transmission Experiments:* The purpose was to select animals believed to be most susceptible and to inoculate into them by various routes heavy suspensions of inclusion-containing tissue. Nodules resulting from injection of aluminum compounds were used as source material. As we have already mentioned, these compounds formed numerous inclusions regularly.

Nodules were removed at intervals of 3, 7, 10, 12, 17 and 24 days. These were ground aseptically with alundum and enough sterile Tyrode's solution to make dilutions from 1:5 to 1:10. After light centrifugation to deposit gross particles including the alundum, the supernatant liquid was used for inoculation. All the injected animals and the original ones bearing nodules were kept in a separate room and isolation precautions were taken. When the nodules were removed, part was taken for section so that it could be determined whether the material actually used for passage contained inclusions. All of these nodules, except the one removed at 3 days, did show inclusions.

A total of 36 mice was given intracerebral injections of 0.03 cc. each of such material. One died of the inoculation; the rest survived for 1 to 2 weeks and during this time showed no clinical evidence of disease. The brains of 3 of these mice were removed for section after 7 to 12 days of observation but no lesions attributable to the action of a virus were found and no inclusions seen.

Fifteen young guinea pigs (10 to 12 days old) were given intracerebral inoculations of 0.05 cc. each. In addition, 3 of them received subcutaneous doses of 1 cc. One died of a perforated colon, and the brains of 4 others were removed for section on the 12th to 15th days. No lesions or inclusions were found. The remaining ones were observed for 2 to 3 weeks but in none did clinical signs of disease develop.

Twelve adult guinea pigs were each given intracerebral inoculations in doses of 0.1 cc., subcutaneous inoculations in doses of 1 cc., and intradermal and subcutaneous inoculations in a pad. The brains of 4 of these were removed for section at intervals of 2 to

12 days. The pads of 6 were removed for section at intervals of 2 to 12 days. Neither brains nor pads showed lesions or inclusions. Barely perceptible transitory indurations appeared in 6 of the animals at the sites of subcutaneous injection. No reactions whatever occurred in others in which the inoculum had been centrifuged more strongly. Skin and subcutaneous tissue at the site of injection were removed for section at intervals of 2 to 12 days from 6 animals. Microscopically the indurations consisted of minute collections of phagocytic mononuclear cells. In 2 of the sections occasional inclusions were found. In these cases tissue had been removed 12 days after subcutaneous inoculation. It was believed that in the instances in which minute indurations were produced these areas contained sufficient chemical which was carried along with the inoculum and set up a slight transient reaction. None of the adult guinea pigs showed clinical signs of infection.

Tissues of animals that had been injected with ground nodule in attempts at transmission of virus were, in turn, ground again and injected into other guinea pigs, thus effecting a second "passage." In 1 test, 2 of the minute, subcutaneous indurations described above were ground with alundum and Tyrode's solution. The supernatant fluid after centrifugation was injected subcutaneously into 6 adult guinea pigs in doses of 1 cc. Four of the 6 developed almost imperceptible indurations that disappeared in about 1 week and the sites of 2 of these were removed at 12 and 13 days for section but no lesion other than some increase in fibroblastic tissue was seen. In another test, brains of 4 young guinea pigs which had received intracerebral injections were ground with alundum and sterile Tyrode's solution. After centrifugation they were injected intracerebrally into 6 adult guinea pigs in doses of 0.1 cc. No signs of infection resulted.

It is therefore apparent from the tests performed on mice and guinea pigs that were inoculated with nodular tissues containing inclusions that no evidence of virus infection was obtained.

*Reinoculation Test:* One might assume that the association of a possible virus with the nodules might set up an immunity so that subsequent injections of aluminum hydroxide would not produce inclusions. An experiment was planned to test this point.

Five guinea pigs were each given 3 inoculations of aluminum hydroxide. One nodule was removed for section from each in 12

to 30 days; these were found to contain the characteristic intranuclear inclusions. On the 41st to 56th day after first injection these animals and a normal control were reinoculated. Twelve or 24 days later nodules derived from the reinjection were removed for section. In addition, some of the first nodules, now 53 days old, were examined.

Inclusions were found in all of the sections of old and new nodules. Consequently no immunity, as postulated, could be shown to exist, since typical new intranuclear structures could be produced, regardless of whether others were present at the time or had previously been demonstrated in the same animals.

*Examination of Submaxillary Glands:* In view of the fact that the inclusions were observed in guinea pigs but not in rabbits and that adult guinea pigs are known to be infected often spontaneously with salivary gland virus, an attempt was made to obtain evidence of its presence. In addition to the transmission experiments already described, this was done by examining for virus inclusions the submaxillary glands of guinea pigs in which nodules produced by aluminum hydroxide had shown inclusions.

Right and left submaxillary glands were removed for section from 4 adult guinea pigs in which the nodules formed by means of aluminum hydroxide had shown many inclusions. The tissues of 2 of these animals revealed occasional intranuclear inclusions typical of the salivary gland virus. The remaining 2 showed none. Two young (10 day old) guinea pigs were given injections of aluminium hydroxide. Nodules were removed on the 7th and 18th days afterwards and also on the latter date (28th day of life) the right and left submaxillary glands were excised for study. Inclusions were found in all 4 of the nodules but in none of the submaxillary glands.

Reference is also made to the fact that in the transmission experiments to young guinea pigs with nodular material, no infection with salivary gland virus was discernible in inoculated animals. Moreover, the characteristic basophilic marginal structures surrounding the intranuclear acidophilic inclusion body of salivary gland disease was not generally seen in the nodules induced by aluminum compounds. It is therefore not likely that the intranuclear inclusions found in the latter represent the bodies brought about by this virus.



In the foregoing experiments attempts were made to detect the presence of any virus that might possibly have been the causal factor in the production of the described intranuclear inclusion bodies. These trials were made by means of animal inoculation of affected tissues, by tests to show whether immunity was induced, and by histopathological studies. What were believed to be the most favorable routes of inoculation and the most susceptible animals were employed. From the results it is apparent that a virus was not demonstrated under these experimental conditions.

### DISCUSSION AND SUMMARY

In the present paper is shown the formation of intranuclear inclusion bodies by the subcutaneous injection of guinea pigs with different aluminum compounds, less often with ferric hydroxide and carbon. They appear to resemble virus inclusions so closely as to be practically indistinguishable from them. One might add that in the instances in which ferric hydroxide and carbon were used they appeared after a prolonged interval and then only for a short period during the height of the reaction. Similar occurrences are known in virus pathology. With aluminum compounds, after the initial period of about 7 days passed, they were observed throughout the duration of the lesion. Finally, special types of cells, phagocytic mononuclear and giant cells, only are involved, and not all foreign body tissue reactions contain the inclusions — barium sulphate, silver chloride, agar, and paraffin have failed.\*

The mechanism underlying the production of these chemically induced inclusions is unknown. Just as with virus inclusions they fail to give the Feulgen (thymonucleic acid) and the masked iron reactions; this is taken as evidence that they do not originate from degenerated nucleoli. Another source might be particles of the introduced foreign substances. In this connection Jacobs,<sup>30</sup> in an article on cell permeability, warns against assuming that a colored substance has not penetrated a cell merely because it cannot be seen. While nuclei are being dealt with and not cells as a whole, a

\* No inference is intended that the aluminum and iron compounds and carbon are the only substances that can possibly give rise to inclusions.

conclusion cannot be reached on this basis as to whether or not the inclusions within the nuclei are particles of the injected substances. This possibility is remote, however, in view of the visible material used, and since in no instance could any part of it be observed to have entered the nucleus.

The point to be stressed is that the intranuclear inclusions produced by chemical means are apparently not associated with a virus; a search which included a number of transmission experiments to animals of the same species in which they were originally produced gave a negative result. In view of this, it is evident that no disease can be classified as a virus disease on evidence of finding in pathological examination of tissues intranuclear inclusion bodies unless it can be proved that they are associated with a virus, that is, unless experimental transmission can be effected.\*

While intracytoplasmic inclusions occurring in the lesions set up by viruses are generally considered as colonies of virus particles — they are commonly found associated with such infective agents of larger size — it becomes more difficult to interpret the significance of virus intranuclear inclusions. One interpretation offered is that changes called forth in the cytoplasm due to virus action might possibly induce changes in the nucleus with the accompanying formation of the intranuclear bodies. From what is here reported, it would appear that a similar sequence of events might follow the engulfing of foreign bodies by cytoplasm, which in turn might influence the nucleus in the manner already described. It is, however, unknown whether or not the structures within the nucleus that appear to resemble so closely those associated with viruses are identical with them in their physicochemical natures.

## CONCLUSIONS

1. Intranuclear inclusion bodies resembling in certain important ways those found in virus diseases were induced by chemical substances such as selected aluminum and ferric compounds and

\* Alundum is used commonly as an aid in grinding virus tissues. It has been shown that alundum itself may induce localized tissue reactions associated with virus-like inclusion bodies. It is therefore conceivable that such inclusions might be mistaken for bodies induced by viruses if care is not taken to centrifuge the alundum out of tissue suspensions to be injected.

carbon but not by others such as barium sulphate, silver chloride, and paraffin, nor by agar.\*

2. No evidence was revealed of a virus infection in association with these inclusions.

3. The results throw light on the interpretation of intranuclear inclusions as they occur in lesions caused by viruses.

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\* After this paper was submitted for publication, intranuclear inclusion bodies similar to those described here were seen in subcutaneous nodules induced in guinea pigs by injection of brain tissue derived from apparently normal animals. This aspect of the problem is now being studied.

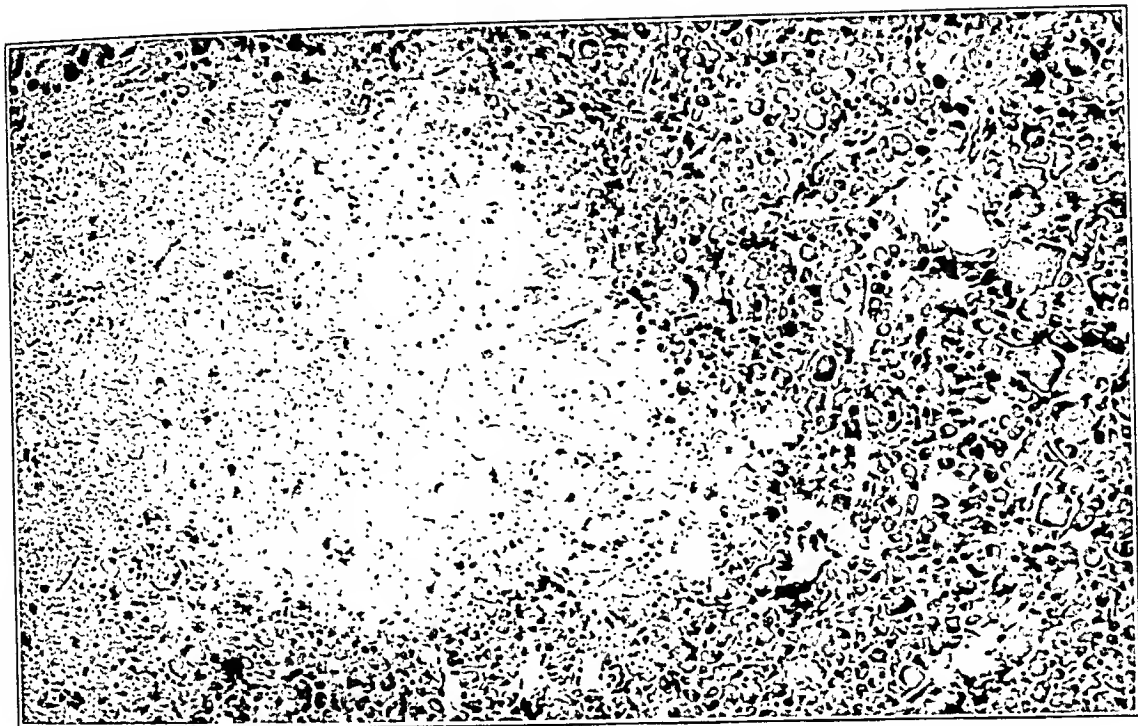
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## DESCRIPTION OF PLATES

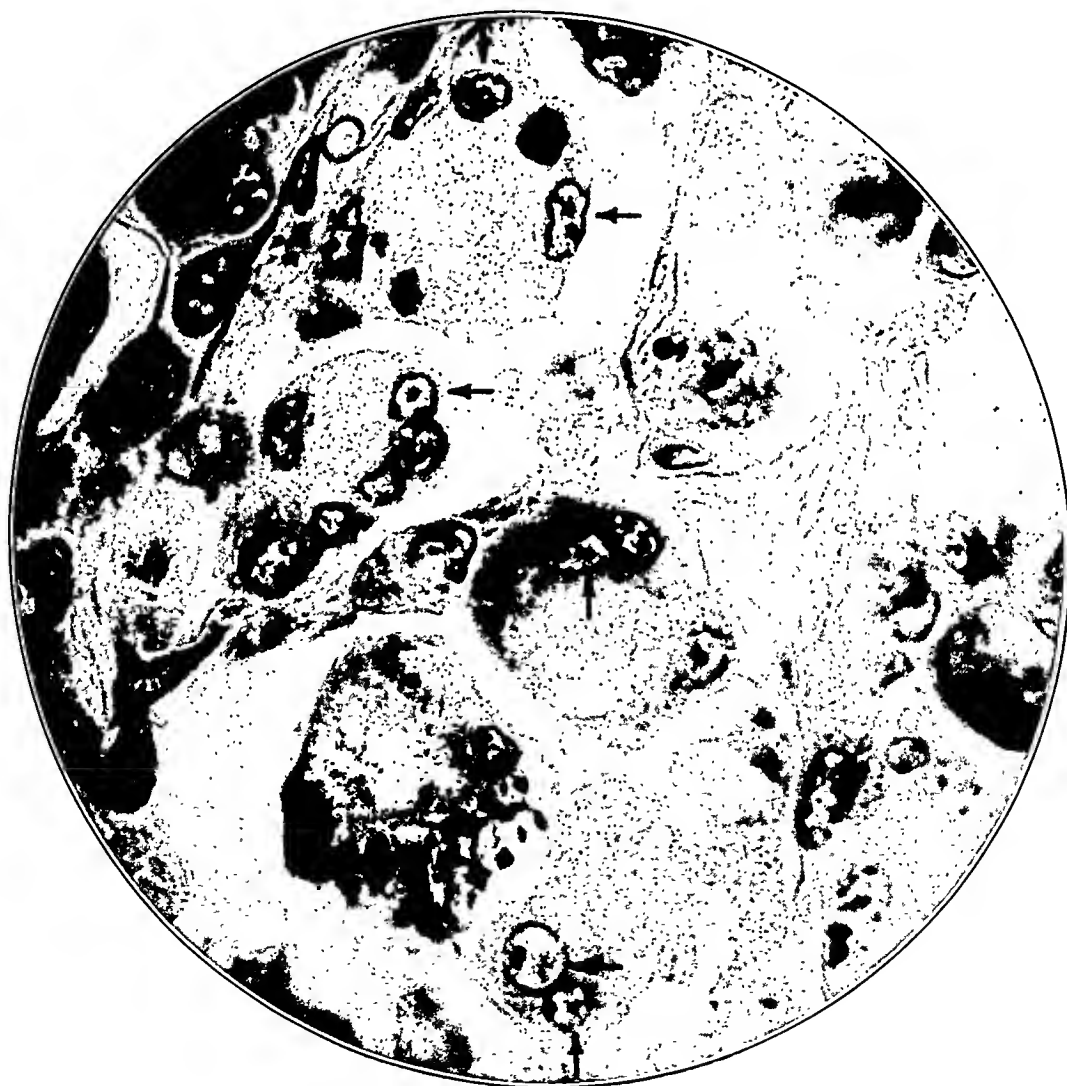
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### PLATE 102

- FIG. 1. Section of a nodule produced by aluminum hydroxide removed from a guinea pig 24 days after injection. The tissue reaction of phagocytic mononuclear and giant cells is shown about the central portion of tissue detritus and chemical. Phloxine-methylene blue.  $\times 100$ .
- FIG. 2. Same section as Fig. 1. Several epithelioid giant cells with peripherally arranged nuclei are present in this field. Arrows point to inclusions. Note that these bodies appear less dense than the nucleoli in their own and adjacent nuclei. Uppermost arrow indicates a large, herpes-like inclusion. Phloxine-methylene blue.  $\times 1000$ .



I



2

PLATE 103

- Fig. 3. Common variety of an inclusion, a single body with margination of the chromatin. In the upper left corner is some amorphous débris and aluminum hydroxide found in the center of a nodule. Phloxine-methylene blue.  $\times 2100$ .
- FIG. 4. A nucleus containing two inclusions. Phloxine-methylene blue.  $\times 2100$ .
- FIG. 5. Giant cell showing inclusions in all of 3 nuclei; 2 other nuclei are shown in shadow because of their deeper sites in the cell. Phloxine-methylene blue.  $\times 2100$ .
- FIG. 6. Large type of inclusion in nodule produced by aluminum hydroxide. Phloxine-methylene blue.  $\times 2100$ .
- FIG. 7. Giant cell in a reaction produced by ferric hydroxide in a guinea pig 18 days after injection. The compound of iron is recognized by its brown color. It distends the cytoplasm but apparently does not penetrate the nuclei. Some of the latter are flattened at the periphery of the cell and contain inclusions which stain red with phloxine. In these nuclei also may be seen several degrees of margination of the blue staining chromatin. Phloxine-methylene blue.  $\times 1500$ .



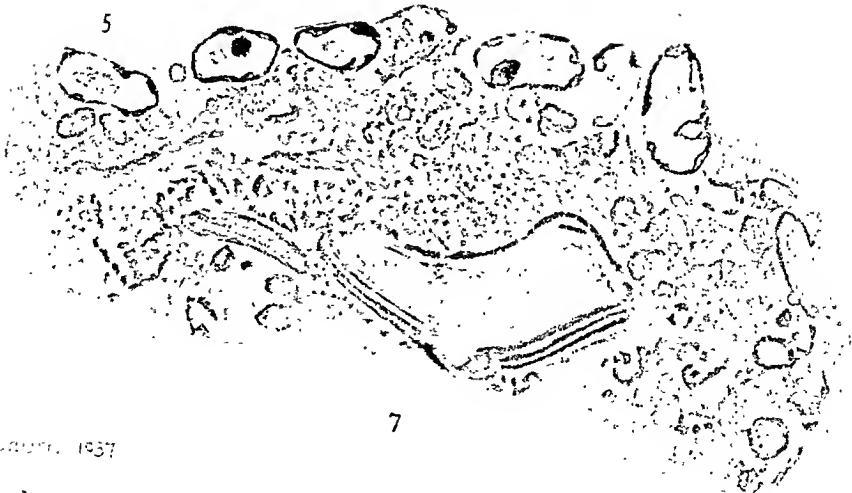
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~ 1937

Olitsky and Harford

Intranuclear Inclusion Bodies





# VIABLE PNEUMOCOCCI AND PNEUMOCOCCIC SPECIFIC SOLUBLE SUBSTANCE IN THE LUNGS FROM CASES OF LOBAR PNEUMONIA \*

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Although the qualitative bacteriology of the lungs in lobar pneumonia is well known, and although some attempts have been made to estimate the number of bacteria by the examination of stained sections, quantitative methods have apparently never been applied to a determination of the number of viable bacteria. Furthermore, although it has been recognized that the consolidated lobes contain large amounts of specific soluble substance, quantitative estimations have not been made.

It was felt that such determinations might furnish information that would be valuable in the study of pneumonic processes during various stages of the disease. The following experiments were conducted with this idea in mind.

## MATERIAL AND METHODS

All material was obtained from routine cases coming to autopsy at the Mallory Institute of Pathology. In each instance the lungs and heart were removed en masse. An area of each lobe was seared and a window was cut away from the sterilized area. A block of tissue weighing from 3 to 10 gm. was removed with sterile instruments to a tared Petri dish. A slice of tissue about 3 mm. in thickness was placed in Zenker's solution. In most instances smears and surface cultures on sheep's blood agar plates were made from the exudate that welled up in the cavity. The pulmonary vessels and bronchi were severed at the hilus of each lung, and the lobes were separated one from another. Each lobe was weighed individually.

Each Petri dish was weighed and the net weight of the block

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of tissue was calculated. With precautions against contaminating the tissue, it was then transferred to a mortar, in which it was thoroughly minced with scissors and then ground for from 4 to 5 minutes with sterile sand. The mixture was washed into a large test tube (200 by 25 mm.) with an amount of Locke's solution equal to five times the weight of the block. After thorough mixing, 1 cc. of the fluid portion was transferred to a tube containing 9 cc. of beef infusion broth, and serial dilutions were made in the same medium; the number of dilution tubes used in each series was governed by the number of bacteria seen in the Gram-stained smear of the original exudate. Pour plates with sheep's blood agar were made, each containing 1 cc. of the desired broth dilutions. Furthermore, 1 or 2 drops of sterile sheep's blood were added to each broth dilution tube. The entire procedure consumed from 3 to 4 hours. Ten drops of chloroform were added to the mixture in each of the large test tubes, which were then closed with rubber stoppers and slowly rotated in a cold room (2-4°C.) for a week or more.

All cultures were incubated at 37°C. and were examined at the end of 24 and 48 hours. The colonies in the pour plates containing moderate numbers were identified and counted. When colonies were typically those of pneumococci, only 1 or 2 were identified by agglutination tests, whereas, when the identity of the colonies was questionable, the organisms from 10 to 20 colonies were tested for bile solubility, inulin fermentation and agglutinability. The numbers of bacteria per milligram of lung tissue were calculated, assigning the value of 1.0 to the specific gravity of the tissue, which value was considered sufficiently accurate for the purposes of calculation. The numbers of bacteria were designated  $\pm$ , +, ++, or +++, depending on whether there were less than  $10^2$ ,  $10^2$  to  $10^3$ ,  $10^3$  to  $10^5$  or greater than  $10^5$  bacteria per milligram, respectively. The broth dilution tubes served as checks on the pour plates, and in several instances where the dilutions used for pour plates were too high, because there were a great deal fewer viable organisms present than had been anticipated, the highest dilution tube revealing growth of pneumococci was arbitrarily considered to have contained one diplococcus. The surface blood agar plates of the exudate furnished rough checks on the numbers and kinds of bacteria.

Samples of from 1 to 2 cc. were removed from the mash tubes in the cold room every 24 or 48 hours until the maximum content of specific soluble substance (SSS) had developed. This usually required a period of from 4 to 7 days. The samples were centrifuged at high speed (12,000 RPM) for about 30 minutes,\* and the clear supernatant fluid, serially diluted in isotonic salt solution, was overlaid on undiluted homologous and heterologous anti-pneumococcic serums contained in tubes with an approximate inside diameter of 3 mm. The highest dilution giving a faint ring with the homologous serum was noted, and the actual amount of SSS in that dilution was calculated, using as a standard the highest dilution of homologous purified SSS \*\* producing a similar ring when overlaid on a portion of the same antiserum. In making serial dilutions one pipette was used for each series. The total amount of SSS in each lobe was calculated, based on the assumption that the precipitinogen taking part in the reaction was entirely SSS and that the tissue examined represented a fair sample of the whole lobe. These rough measurements and rather loose assumptions are considered valid only in demonstrating the very wide differences that were found to exist in the amount of SSS contained in the lobes at various stages of consolidation. In 2 cases the SSS was determined in the liver, spleen, blood serum, urine and pleural fluid. For the liver and spleen the method was identical with that for the lungs. For the blood serum, urine and pleural fluid, simple dilutions in isotonic salt solution were employed.

The Zenker-fixed tissue from the lungs was embedded in paraffin and sections were cut approximately  $4\ \mu$  in thickness. One set of sections was stained with phloxine and methylene blue and another with Rudinkoff and Stawsky's modification<sup>1</sup> of Brown and Brenn's method for differential staining for Gram-positive and negative bacteria. With the optical system used — Zeiss 90 objective and 10 x ocular — each field was equal to approximately  $1/14,000$  of a cubic millimeter. The sections were carefully

\* This was done in a No. 1 centrifuge (International Equipment Co.) with a high speed attachment. The head was always chilled in a cold room before use to prevent overheating.

\*\* These were kindly supplied by Dr. John Enders, Department of Bacteriology, Harvard Medical School, and Dr. Maxwell Finland, Thorndike Memorial Laboratory, Boston City Hospital.

TABLE I — *Histological, Bacteriological*

Clinical, bacteriological and statistical data		Lobe	Gross pathology	Histological			
				Pneumococci			
				Alveoli		Bronchi	
				Free	Phag.	Free	Phag.
Case 10 W ♂ 43 Lobar pneumonia Type I — 5 days duration Autopsy — 3 hrs. PM	Sp (3) Pk I, BMC VB (A) Pk I PI F (A) L (400 cc.) Pk I L (A) BC S (A) BMC Antiserum — 60 cc. (3, 4)	RUL	Mod. late consol.	o	+	o	+++
		RML	Sl. edema	+	++	o	+++
		RLL	Sl. edema	+++	++	+++	+++
		LUL	Late consol.	+	o	o	o
		LLL	Sl. edema (RBC)	+	o	o	o
Case 4 W ♂ 39 Lobar pneumonia (Cardiac disease) Type I — 6 days duration Autopsy — 17 hrs. PM	Sp (6) Pk I VB (A) Pk I Antiserum — 5 cc. (6)	RUL	Mod. late consol.	o	o	o	o
		RML	Normal	o	o	o	+++
		RLL	Mod. late consol.	o	o	o	o
		LUL	Normal	+	+	o	o
		LLL	Mod. late consol.	+	o	++	++
Case 2 W ♂ 32 Lobar pneumonia (Alcoholism) Type I — 8 days duration Autopsy — 4 hrs. PM	Sp (4) Pk I PI F (A) R (200 cc.) o	RUL	Late consol.	o	o	o	o
		RML	Mod. late consol.	o	o	o	o
		RLL	Mod. late consol.	o	++	o	o
		LUL	Normal	o	o	o	o
		LLL	Normal	o	o	o	+
Case 11 W ♂ 74 Lobar pneumonia (Alcoholism) Type I — 8 days duration Autopsy — 5 hrs. PM	Sp (2) Pk I VB (A) Pk I L (A) BW S (A) BW, BC	RUL	Congestion (RBC)	o	o	o	++
		RML	Congestion (RBC)	..	..	..	..
		RLL	Congestion (RBC)	o	o	+++	+
		LUL	Mod. late consol.	+	++	++	++
		LLL	Mod. late consol.	+	+++	++	++
Case 3 W ♀ 70 Lobar pneumonia (Cardiac disease) Type I — 12 days duration Autopsy — 10 hrs. PM	VB (8) Pk I	RUL	Normal	o	+++	o	+++
		RML	Absent	..	..	..	..
		RLL	Late consol.	o	++	o	+++
		LUL	Normal	o	++	++	+++
		LLL	Early consol. (RBC)	o	+++	+++	+++
Case 1 W ♂ 18 Lobar pneumonia Type II — 13 days duration Autopsy — 3 hrs. PM	Sp (4) Pk II VB (4, 9, 11) Pk II PI F (A) R (500 cc.) o L (700 cc.) o Antiserum — 225 cc. (4, 5)	RUL	Lymphangitis	o	o	o	++
		RML	Late consol. (lymphangitis)	+	o	o	o
		RLL	Late consol.	++	+++	o	+++
		LUL	Normal	o	o	o	o
		LLL	Mod. late consol. { lym'tis RBC }	o	+++	o	o
Case 6 W ♀ 72 Lobar pneumonia Type III — 8 days duration Autopsy — 11 hrs. PM	Sp (7) Pk III VB (8) Pk III	RUL	Mod. late consol.	o	+++	o	+++
		RML	Early consol.	..	..	..	..
		RLL	Early consol.	++	+++	o	o
		LUL	Congestion (RBC)	++	o	++	o
		LLL	Late consol.	++	+++	+++	+++
Case 8 W ♀ 50 Lobar pneumonia Type III — 8 days duration Autopsy — 4 hrs. PM	Sp (6) Pk III VB (7) Pk III PI F (A) R (500 cc.) o	RUL	Normal	o	o	o	o
		RML	Mod. late consol.	o	o	o	o
		RLL	Early consol.	o	+++	o	+++
		LUL	Normal	o	o	+++	++
		LLL	Sl. edema	o	o	++	++
Case 9 W ♂ 51 Lobar pneumonia Type III — 8 days duration Autopsy — 12 hrs. PM		RUL	Early consol.	o	+++	o	++
		RML	Late consol.	+	+	o	+
		RLL	Resolving consol.	+++	o	+	o
		LUL	Congestion and edema	+	o	o	o
		LLL	Congestion and edema	+++	o	o	o

*General Data:* Sp = sputum, VB = venous blood, PI F = pleural fluid (R = right, L = left), L = liver, S = spleen, RBC = red blood corpuscles, Pk = *Pneumococcus*, BMC = *B. mucosus capsulatus*, BW = *B. Welchii*, and BC = *B. coli*. Numbers in parentheses represent day of disease; A = autopsy.

*Lobe:* Bold face type represents the primarily involved lobe.

and Serological Findings in All Cases

bacteriology	Cultural bacteriology		pH	Soluble specific substance		Appreciable numbers of viable pneumococci			
Other bacteria	Pneu-mo-cocci	Other bacteria		Lungs	Other organs	Norm. lobes	Cong. or cd. lobes	Cons. lobes	Bron-chi
BMC+++ FGPB++ GNB++ BMC++	o ++ ++ ++R o	BMC ± BMC+++ BMC+++ SV++ BMC+++ SV++ BMC+++	.. .. .. .. ..	mg. 800 20 400 1000 60	mg. Pl F 200 B 40 U 4 L & S < 10 Total - 2500		 + +  o	 o  +	
GPB++ GPC++	± ± o + o	SA ± SA ± SV ± SA++ SV+ SA ± SV ±	5.7C 6.0 5.5 6.3 6.0	100 1 400 3 100	B 4 U 1 L & S 11  Total - 600	  o o		  o o	   +
	± ± ± ± ±		7.2C 7.0 6.5 7.6 7.4	20 80 400 1 1	    Total - 500	   o o		  o o o	
FGPB++ FGPB++ GPC++ GNB++	++ .. o ++ +	BMC ± BMC ± BMC ± SA ±	6.9E .. 7.4 6.9 7.1	↓ 20 ↑ 1600 200	    Total - 1800		 o o	  o o	 + +
FGPB++ FGPB++ GPC++ GNB++	+ .. o ++ +	Pk (XXX) +	7.0C .. 6.2 7.0 6.9	1 .. 23 1 7	    Total - 30	 o o		  o o	 + +
GPC++	± ± ± ± ±		7.4C 6.3 7.5  7.4 6.1	9 20 600  1 600	     Total - 1200	 o  o		  o o	
GPC++ GPC+++ GPC+++ GPB++ GNB++	o ± ± +++ ±	SA++ SA++ SA+++ SA ±	.. .. .. .. ..	5800 200 500 80 1800	    Total - 8400		   +	  o o o	
GNC+++ GNC+++ GPC+++ GPB++ GNB++	± ± ++ ++	SV ± SV+ SV++	.. .. .. .. ..	? 200 300 500 700	    Total - 1700	 o o		  o o	   +
FGPB+++ GPC+++ GNC+++ FGPB+++ FGPB+++ GPC+++ HI+++	± ++ ++ ++	SV+++ SA++ SH++ SV+++ SA++ SV+++ SA+++	6.0E 6.1 6.7 7.2 7.0	600 200 20 5 20	    Total - 850		   +	  o +	

Histological Bacteriology: BMC = bacilli similar to *B. mucosus capsulatus*, FGPB = fat Gram-positive bacilli, GNB = Gram-negative bacilli, GNC = Gram-negative cocci, GPC = Gram-positive cocci, and HI = bacilli similar to *H. influenzae*.  
Cultural Bacteriology: BMC = *B. mucosus capsulatus*, SV = *Streptococcus viridans*, SA = *Staphylococcus aureus*, Pk = *Pneumococcus*, SH = *Streptococcus hemolyticus* and R = rough.  
pH: C = colorimetric and E = electrometric.  
SSS: B = Blood, U = Urine.

studied. The predominant type of consolidation in each lobe was observed and noted. Those designated "early" showed an exudate in the alveoli that consisted, chiefly, of neutrophilic polymorphonuclear leukocytes embedded in a meshwork of fine fibrin strands; thromboses in the small blood vessels of the alveolar walls were frequently noted. In those considered "late," the exudate contained few to many mononuclear leukocytes; the polymorphonuclear leukocytes as a rule showed degenerative changes, the fibrin plugs were shrunken and partly digested, and the alveolar walls were thickened because of dilatation of the capillaries and proliferation of cells lining the alveoli.

The numbers of free and phagocytosed pneumococci and other bacteria in the alveoli and in the bronchi were designated +, ++ or +++ depending on whether there were a few in many fields, a few in almost all fields or moderate numbers to many in almost all or all fields, respectively.

These designations were roughly comparable to those used for the cultural results. For example, 1 pneumococcus in 14 fields would be called a "+" histologically and, taking into account the volume of one field (1/14,000 cmm.), this would mean 1000 pneumococci per cubic millimeter which, as has been previously noted, would be called a "+" culturally.

The hydrogen ion concentrations were determined colorimetrically or electrometrically, using small amounts of relatively fresh lung suspensions.

## RESULTS

All of the findings in each case are given in Table I, together with a certain amount of clinical and pathological data.

*Pneumococci:* There is fairly good agreement between the numbers of free pneumococci observed in the sections and those recovered culturally. It should be realized that many of the former may not have been alive at the time the sections were taken and counts of viable organisms were made and that many of the latter may have come from the exudate in the bronchi or bronchioles. Provided, however, due allowance is made for contamination from this extraparenchymal exudate, based on a careful study of the stained section, the numbers recovered culturally probably

represented fairly accurately the viable pneumococci in the lung substance at the time of the autopsy.

As shown in Table II, appreciable numbers of viable pneumococci were recovered from only 3, 13 per cent, of the consolidated lobes, namely, from 1 lobe in Case 10, from which only rough variants were cultured, and from 2 lobes in Case 9, in which the consolidation was very late. In the latter case, the presence of very large numbers of pneumococci in the congested and edematous lobes suggests the possibility that the consolidated lobes may have become reinfected shortly before death. The normal lobes contained no viable pneumococci that could not be justly accounted

TABLE II

*Summary of Incidence of Viable Pneumococci in All Lobes*

Condition of lobe	None to few pneumococci	Moderate numbers to many pneumococci	Probable bronchial contamination	Corrected incidence of appreciable numbers of pneumococci		
				Lobes		Per cent
				Negative	Positive	Positive
Normal	6	4	4	10	0	0
Congestion or edema or both	1	8	3	4	5	56
Consolidation	20	4	1	21(24*)	3(0*)	13(0*)

\* If exceptions, noted in the text, are taken.

for by contamination from bronchial or bronchiolar exudate, whereas they were found in 56 per cent of the lobes with congestion or edema or both. It should be noted that extravasated serum and a certain number of red blood cells were always found in the alveoli of lobes revealing marked congestion.

Variation in the interval between death and autopsy had no evident effect on the above findings.

*Other Bacteria:* Bacteria, other than pneumococci, were seen in the sections or recovered in culture from all but 2 of the 9 cases. The incidence is shown in Table III.

The 2 cases (No. 9 and No. 10) that had the greatest amount of edema in the non-consolidated lobes showed by far the greatest number of bacteria other than pneumococci.

*Phagocytosis:* Phagocytosed bacteria were seen in nearly all of the preparations, most commonly in the exudate in the bronchi



TABLE III  
Summary of Incidence of Bacteria Other than Pneumococci in All Cases

Sections		Cultures	
Description of bacteria	Number of cases	Organisms	Number of Cases
Gram-positive cocci	5	<i>Streptococcus viridans</i>	4
Gram-negative bacilli, slender	4	<i>Staphylococcus aureus</i>	4
Gram-positive bacilli, fat ( <i>B. Welchii</i> )	3	<i>B. mucosus capsulatus</i>	2
Gram-positive bacilli, slender	3	<i>Streptococcus hemolyticus</i>	1
Gram-negative diplococci	2	<i>Pneumococcus</i> , Type XXX	1
Gram-negative bacilli, fat ( <i>B. mucosus capsulatus</i> )	1	<i>Pneumococcus</i> , rough, variant	1
Gram-negative bacilli, short and slender ( <i>H. influenzae</i> )	1		

or bronchioles. With the exception of 1 case, the phagocytic cells were chiefly polymorphonuclear leukocytes. In Case 1, treated with 225 cc. of serum, death occurred on the 13th day of the disease; practically no pneumococci were recovered from the cultures and the majority of the phagocytosed pneumococci were contained in mononuclear leukocytes. In only a relatively few instances were free pneumococci seen in the alveoli in the absence of phagocytosis.

*Hydrogen Ion Concentration:* The color of the lung suspensions interfered considerably with the determination of the hydrogen ion concentration by the colorimetric method. Furthermore, no attempt was made to protect samples against loss of carbon dioxide. Although the figures listed almost certainly do not represent the actual hydrogen ion concentrations of the different lobes during life, they may, in the absence of data from living cases, serve to furnish an idea as to the relative reactions of the different lobes. In general, the consolidated lobes showed a higher concentration of hydrogen ions than the normal or congested and edematous lobes.

*Specific Soluble Substance (SSS):* The amounts of SSS in the lungs were large. In general, the greatest amounts were found in the moderately old, consolidated lobes, and the total amounts in the lungs were more frequently large in cases of relatively short duration.

In Case 3, in which death occurred on the 12th day of the disease, the amount of SSS in the consolidated lobes was negligible; in view of the findings in other lungs showing earlier consolidation, it seems probable that it had been present previously in large amounts and had been almost completely removed or destroyed. The same is true for the resolving right lower lobe in Case 9.

Appreciable amounts of SSS were found in the seropurulent pleural fluids in Case 10. The amounts in the blood serum, the urine, the liver and the spleen in Cases 4 and 10 were negligible.

## DISCUSSION

Loeschcke,<sup>2</sup> in 1931, published his views on the genesis, course and mechanism of spread of lobar pneumonia, which were based on a systematic gross and microscopic study of about 90 lungs,

fixed *in toto*, from cases of the disease. His division of the disease into five stages, with a description of the transition from one stage to the next, is considered, in this laboratory, to be more acceptable than the early classical descriptions. According to him, there is, first, hyperemia of the alveolar walls, and the alveoli are filled with edema fluid and innumerable numbers of pneumococci. This is followed by the second stage, in which there is migration of the polymorphonuclear leukocytes and, to a less extent, of "alveolar epithelial cells" into the alveoli, with or without extravasation of red blood cells. In the third stage, the alveoli are completely filled with an exudate consisting chiefly of polymorphonuclear leukocytes in a fine meshwork of fibrin threads, few free pneumococci are present, the alveolar walls are very ischemic and, frequently, there is evidence of thrombosis of the small blood vessels. In the fourth stage, the fibrin network shrinks down, the fibrin threads become coarser, the blood vessels in the alveolar walls refill, extravasation of red blood cells into the alveoli usually occurs, the polymorphonuclear leukocytes are markedly decreased in numbers and show extreme degenerative changes, and there is a regeneration of the "alveolar epithelial cells." Finally, in the fifth stage, the polymorphonuclear leukocytes have disappeared and the remaining fibrin is in the form of tiny, compact plugs that contain and are surrounded by "alveolar epithelial cells," which eventually digest the last remains of the pneumonic process.

The findings in the lungs in this series of cases were in accord with Loeschcke's description of the sequence of events and were essentially the same as those described by Robertson and Uhley<sup>3</sup> in human pneumonic lungs, by Robertson, Coggeshall and Terrell<sup>4</sup> in experimental pneumonia in dogs, and by Rhoads and Goodner<sup>5</sup> in dermal pneumococcus infection in rabbits.

Opie<sup>6</sup> studied the antitryptic substance in blood serum which prevents the digestion of fibrin by leukocytes. This antienzyme was found to be active only in an alkaline medium. Kline and Winternitz<sup>7</sup> demonstrated marked impairment in circulation in the consolidated lung, observing that only enough blood is allowed to seep through the vessels to nourish the alveolar walls, with the result that very little serum escapes into the alveoli and the autolysis of the exudate by the leukocytes is unhindered. Lord<sup>8</sup> demonstrated in the lung the presence of a proteolytic

enzyme active in a weakly alkaline medium, and a peptone splitting enzyme active in an acid medium. He expressed the belief that, as the acidity in the consolidated lung increases, the former enzyme becomes inactive and the latter activated. In this series of autopsy cases it was noted that the fibrin plugs in the alveoli adjacent to the septa carrying the bronchi and larger blood vessels were much more dense than those more centrally located. The presence of anti ferment in the connective tissue about the septa or the ability of the septal tissues to maintain a slightly alkaline reaction in the contiguous alveoli could, either singly or in combination, explain the relatively slight degree of fibrinolysis noted in the adjacent alveoli.

The outstanding feature of the cultural results was the failure to recover appreciable numbers of pneumococci from the consolidated lobes, regardless of whether the process was early or late. The rapid disappearance of pneumococci from pneumonic processes was long ago noted by Babes<sup>9</sup> and Weichselbaum.<sup>10</sup> Wadsworth<sup>11</sup> reported that he was able to cultivate very few pneumococci from lungs revealing gray hepatization. Similar observations were made by Rosenow,<sup>12</sup> who further showed, by means of lung punctures, that with the onset of resolution in a given area, pneumococci failed to reappear in cases with ultimate recovery, whereas reinvasion in increased numbers occurred in cases with fatal termination. Such was evidently the occurrence in Case 9 in this series, wherein 2 lobes showing very late consolidation contained considerable numbers of viable pneumococci.

The cause of the early loss of viability of the microorganisms is uncertain. Phagocytosis by polymorphonuclear cells apparently is a factor, although destruction of pneumococci or suppression of growth is known to occur *in vivo*, under certain circumstances, in the absence of marked phagocytosis. On the other hand, Robertson and Uhley<sup>3</sup> state that effective destruction of pneumococci does not occur during the polymorphonuclear stage, but rather during the subsequent stage of macrophage proliferation. They succeeded in culturing pneumococci from areas in the former stage of consolidation and noted that the microorganisms, histologically, appeared in good condition, even after phagocytosis by polymorphonuclear leukocytes. From these findings they conclude that phagocytosis

by polymorphonuclear cells does not represent an effective mechanism for disposing of the bacteria, but that widespread destruction occurs during the stage of macrophage proliferation. In this small series of cases marked diminution in the numbers of viable pneumococci occurred during the stage of phagocytosis by polymorphonuclear leukocytes. Although the possibility must be borne in mind that the pneumococci may die more rapidly postmortem in this stage of consolidation than microorganisms in earlier lesions, marked alteration in the cultural picture by postmortem changes seems improbable in those cases examined as early as 3 hours after death; and, since the autopsies performed after longer intervals yielded essentially similar results, it was felt the time factor did not introduce a variable of prime significance.

The relative importance of the polymorphonuclear leukocytes and other factors, specific or non-specific, in causing the early removal of pneumococci from lesions is highly uncertain. There may be considerable variation in the bactericidal power of the polymorphonuclear leukocytes in the lungs in different cases, which is comparable to the individual differences found in the phagocytic activity of cells of the circulating blood of animals by Robertson and Sia,<sup>13</sup> and of man by Sutliff and Rhoades<sup>14</sup> and by Ward.<sup>15</sup> The fact that marked phagocytosis occurred in every case in this series was, by itself, considered noteworthy, for in some instances the amounts of SSS were very large. Rosenow<sup>16</sup> described a soluble substance in pneumococcus autolysates, which inhibited the action of pneumococcic opsonin. Sia,<sup>17</sup> Wadsworth and Sickles,<sup>18</sup> Ward<sup>19</sup> and others have studied the inhibitory effect of SSS upon *in vitro* phagocytosis. Cole<sup>20</sup> established the fact that the soluble substances of pneumonic exudates neutralized the effects of antipneumococcic immune bodies. Virulent pneumococci are not phagocytosed by polymorphonuclear leukocytes except in the presence of immune substances, and it is difficult to understand how such substances remain active in the presence of the very large amounts of supposedly unneutralized SSS. One is led to seek for possible explanations.

Ward<sup>21</sup> showed that, whereas SSS exerted a specific inhibitory effect on phagocytosis, this effect could be neutralized by

amounts of homologous antiserum far less than that required to produce any detectable precipitation and that the addition of an excess of antiserum resulted in the formation of a precipitate, which was ingested by the leukocytes, thus interfering with phagocytosis apparently for mechanical reasons. Furthermore, by *in vitro* experiments with type III pneumococci he showed that homologous convalescent human serum was able to neutralize the inhibitory effect of SSS and sensitize pneumococci in concentrations that produced no precipitation, whereas in similar experiments with antipneumococcic horse serum effective concentrations yielded a precipitate, which was itself antiphagocytic. The findings of Ward suggest that in lobar pneumonia immune substances may be elaborated in sufficient concentration both to overcome the inhibitory action of the SSS and to sensitize the pneumococci although the mechanism of a response that would result in as rapid and as effectual phagocytosis as was observed in this series of cases is difficult to conceive.

Another, and possibly a more rational, explanation is the assumption that some non-specific physicochemical factor in the exudate kills the pneumococci, rendering them more susceptible to phagocytosis. Rich<sup>22</sup> has expressed the belief that such factors are extremely important for the control of infection in many inflammatory foci. The only such factor investigated in this series of cases was the hydrogen ion concentration. As has already been stated, the figures obtained postmortem cannot be assumed to represent the exact pH of the pneumonic exudate *in vivo*. However, since the pH of the consolidated lobes was, in general, conspicuously lower than that of the unconsolidated lobes, it suggests the possibility, advanced by Lord,<sup>23</sup> that a factor in the death of the bacteria may be an increase in the intra-alveolar hydrogen ion concentration, resulting from rapidly dying and autolyzing leukocytes and impaired circulation in the alveolar walls. Lord and Nye<sup>24</sup> have shown that pneumococci are extremely sensitive to slight changes of acidity. The important observation of Rous<sup>25</sup> is noteworthy in this regard. He ascertained that the reaction of granules in living macrophages is pH 3 or less, and called attention to the lethal action that such a high hydrogen ion concentration might be expected

to exert on phagocytosed pneumococci. That changes in the hydrogen ion concentration have an important bearing on the type of cellular reaction and enzymatic activity is emphasized by Rich<sup>22</sup>; thus, indirectly, the viability of the pneumococci may be affected by a lowering of the pH.

With regard to the claim of Robertson and Uhley<sup>3</sup> that the mononuclear phagocyte is actively associated with the removal or killing of viable pneumococci, a study of the cases in this series makes it seem much more likely that the majority of pneumococci are dead and phagocytosed before the mononuclear cells appear in large numbers, that the latter are concerned with the removal of foreign material, chiefly fibrin and dead polymorphonuclear leukocytes, and that the pneumococci seen in mononuclear cells mainly represent those contained in ingested polymorphonuclear cells. Further contributory evidence of the scavenger function of these mononuclear cells is furnished by their marked proliferation in those alveoli, previously noted, that lie adjacent to the septa and that contain dense plugs of fibrin.

Two of the most serious prognostic conditions in lobar pneumonia—positive blood culture and spread of the lesion—appear to be intimately related to lack of uniform consolidation. In the first stage of lobar pneumonia, according to Loeschcke,<sup>2</sup> there is edema and multiplication of pneumococci within the alveoli, to which the body reacts with a rapid mobilization of enormous numbers of polymorphonuclear leukocytes. A uniform, lobar type of consolidation results, and the disease process becomes localized. Isolated foci of alveoli containing pneumococci and edema fluid may persist in areas other than those of consolidation. In these the influx of leukocytes is much more gradual; it is possible that this delay favors the extension of the consolidation and the development of bacteremia. The mechanisms involved in the localization of the pneumonic process are by no means clear. Wadsworth<sup>26</sup> actively immunized rabbits with killed whole pneumococci and found that the introduction of virulent pneumococci into the lungs of animals so immunized did not give rise to an early general infection, but that the microorganisms remained localized in the lungs, where, if the immunization was not of too high a degree, a disease process

was produced that was similar to lobar pneumonia in man. Opie,<sup>27</sup> in a general discussion of inflammation and immunity, stressed the role of hypersensitiveness in bringing antibodies to the site of attack and fixing bacteria at the site of entry so that they cannot enter the blood stream. Lauche<sup>28</sup> has speculatively linked allergy with immunity in the pathogenesis of lobar pneumonia, holding that the character of the disease is the result of hyperergy and an associated partial immunity and that patchy bronchopneumonia develops because of the lack of such sensitivity and in the corresponding absence of as high a degree of immunity. Lauche's hypothesis is not supported by proof. Robertson, Coggeshall and Terrell,<sup>4</sup> however, have shown in experimental pneumonia in dogs that animals with uniform consolidation only rarely develop blood stream invasion and that in animals developing a generalized infection the consolidation is usually patchy. Furthermore, Sutliff and Finland<sup>29</sup> have published statistics indicating a relatively high mortality in cases of bronchopneumonia incited by pneumococci of a given type compared with that in cases of lobar pneumonia of the same type.

The intravenous injection of homologous antipneumococcic serum is quite generally believed to be therapeutically valuable in preventing or controlling the spread of the pneumonic lesion, but its possible action within areas of consolidation has been subject to much controversy. Cole,<sup>30</sup> in an early article on the serum treatment of pneumonia, observed that when treatment was commenced early no extension of involvement of the lung occurred, but that there was no tendency for the already consolidated portions to resolve under the influence of serum therapy. Hunnicutt and Sutliff,<sup>31</sup> on the basis of precipitation tests, concluded that penetration of the consolidated lobe by therapeutic serum occurs, but that there is no evidence to show that the serum reaches the microorganisms in the already formed pneumonic exudate. In studying the circulation in the pneumonic lungs of rabbits, Kline and Winternitz<sup>7</sup> found that trypan blue readily reached the alveoli when instilled into the bronchi but not when injected intravenously; they attributed this failure to the marked impairment of circulation due to the wide distribution of capillary thrombi.

Even were it proved that the serum can penetrate into the



exudate, its effectiveness in the presence of large amounts of SSS would be exceedingly problematical. The establishment of an excess of antibody would seem to be impossible. By determining the amount of antiserum required to precipitate completely the purified polysaccharide in a given dilution of this substance, calculations were made of the amount that would be required to precipitate the SSS from the corresponding concentration in the lung suspensions. On the basis of these figures it was estimated that in Case 10, for example, 60 liters of the highly potent type I antiserum that was used in testing for the SSS would have been necessary to precipitate completely the specific carbohydrate in both lungs, provided, of course, that the SSS in the sample of lung tissue obtained from each lobe represented a fair sample of the total amount in the whole lobe. The possibility must not be overlooked that in cases with ultimate recovery, the concentration of SSS may never reach such a high level. In the light of what is known regarding the inhibitory action of precipitates upon phagocytosis, the establishment of an excess of antibody would appear to be definitely injurious. The mode of action of therapeutic horse serum remains a fertile field for investigation; the fact is clear, nevertheless, that to be effective therapeutic serum must be given early before extensive consolidation has occurred.

It is known that cases of lobar pneumonia may result fatally in spite of the fact that the pneumococcic infection has been largely overcome. This was true in 2 of the 6 cases due to type I pneumococcus, wherein no appreciable numbers of bacteria were cultivated from the lungs. In both Cases 1 and 2 there was no obvious reason for death other than extensive involvement and, in explaining the fatal outcome, the possibility of secondary causes of death resulting from the "toxemia" must be seriously considered, as, for example, profound circulatory disturbances (Atchley,<sup>32</sup> Hitzig *et al.*,<sup>33</sup> and Warfield<sup>34</sup>).

Friedlander bacilli were cultivated from 2 of the 9 cases and were in sufficiently large numbers to be considered as a contributory cause of death. From the experience gained in routine autopsy bacteriology in this laboratory the feeling has arisen that this bacillus and certain other bacteria are usually, if not always, secondary invaders.

## SUMMARY

1. Quantitative bacteriological methods have shown that viable pneumococci are extremely scarce in the consolidated lobes of lungs from cases of lobar pneumonia.

2. A quantitative serological method has demonstrated that homologous soluble specific substance is present in such lobes in high concentration and that, as a rule, the moderately old, consolidated lobes contain the largest amounts.

3. Histological examination of sections of the same lobes has shown that the majority of the pneumococci are phagocytosed by polymorphonuclear leukocytes.

4. Certain aspects of the pathology and bacteriology of lobar pneumonia are discussed.

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## THE RÔLE PLAYED BY RHEUMATIC FEVER IN THE IMPLANTATION OF BACTERIAL ENDOCARDITIS \*

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According to Perry,<sup>1</sup> the first recorded clinical and pathological findings in a case of bacterial endocarditis were by Lancisi in 1707. A century later Wells,<sup>2</sup> Corvisart,<sup>3</sup> and Bouillaud<sup>4</sup> clearly described the small excrescences (verrucae) characteristic of rheumatic heart disease. The first attempt at differentiation between these conditions seems to have been made by Ormerod<sup>5</sup> in 1851. In the following year Kirkes<sup>6</sup> described the prolonged form of infectious endocarditis, and in 1885 Osler<sup>7</sup> recorded the important observation that valves which were the seat of "malignant endocarditis" frequently show signs of previous damage. Toward the end of the nineteenth century Kelynack's<sup>8</sup> statistical studies on the frequency with which the valves in bacterial endocarditis show evidence of preexisting lesions marked a considerable advance in this field. In 1903 Glynn<sup>9</sup> reported that 60 per cent of his 65 cases of bacterial endocarditis gave a previous history of rheumatic fever. The succeeding years have been replete with classical contributions to the disease which Libman has termed "subacute bacterial endocarditis" and to the knowledge of which he has made notable contributions.<sup>10-20</sup>

In their excellent monographs on bacterial endocarditis Blumer,<sup>21</sup> Thayer,<sup>22</sup> and Perry<sup>1</sup> emphasized the frequent association and the etiological relation of rheumatic valvular disease to these conditions. In a study of their statistics and of other pertinent reports (Libman,<sup>10</sup> Horder,<sup>23</sup> Billings,<sup>24</sup> Coombs,<sup>25</sup> Clawson,<sup>26</sup> Clawson, Bell and Hartzell,<sup>27</sup> Morrison,<sup>28</sup> Swift,<sup>29</sup> MacCallum,<sup>30</sup> and Davis and Weiss<sup>31</sup>) it becomes clear that previous rheumatic alterations are to be found in at least 50 per cent of hearts that are the seat of bacterial endocarditis. In 1923 Lib-

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man<sup>17</sup> described the simultaneous occurrence of active rheumatic fever and subacute bacterial endocarditis in the same hearts. In 1929 Clawson<sup>32</sup> drew attention to the occurrence of Aschoff bodies in 45 per cent of his 60 cases of subacute bacterial endocarditis and assumed a common etiology for both. In 1933 Saphir and Wile<sup>33</sup> reported 10 cases of subacute bacterial endocarditis in children and adolescents, all of which showed Aschoff bodies in the heart. These authors were of the opinion that these lesions represented two distinct entities. In a recent communication Gross<sup>34</sup> presented evidence that indicates the majority of bicuspid aortic valves seen in adults owe their deformity to a rheumatic process. Since these altered aortic valves are known to be peculiarly disposed to bacterial endocarditis (Paget,<sup>35</sup> Abbott,<sup>36, 37</sup> Osler,<sup>38</sup> Lewis and Grant<sup>39</sup>) the close association of these two conditions was again emphasized. In 1935 VonGlahn and Pappenheimer<sup>40</sup> came to the conclusion that infection of the cardiac valves with non-hemolytic streptococci in rheumatic patients is due to the implantation of the bacteria on unhealed rheumatic vegetations. This view is somewhat similar to that expressed by Königer in 1903.<sup>41</sup> VonGlahn and Pappenheimer's evidence for this conclusion, based on an examination of 26 consecutive cases of subacute bacterial endocarditis, is summarized by these authors as follows:

"1. Vegetations histologically identical with those in rheumatic endocarditis and not containing bacteria are found (a) on the same valve as the bacterial vegetations, (b) on other valves on which there are no vegetations containing bacteria, and (c) on the auricular wall.

2. Aschoff bodies in the myocardium which are taken to indicate active rheumatic disease are found in practically the same proportion of cases of subacute bacterial endocarditis as of uncomplicated rheumatic cardiac disease.

3. Types of bacterial endocarditis other than that due to non-hemolytic streptococci may be engrafted on active rheumatic vegetations. This is a cogent argument against the view that the two types of lesions are a response of different intensity to the same infective agent, unless we dispense with current views as to the histological specificity of the rheumatic reaction."

In a series of publications on the life cycles of various cardiac lesions in the rheumatic heart, Gross *et al.*<sup>42-51</sup> have demonstrated widespread stigmata by which it is possible to recognize an underlying rheumatic process, even though it be extinct. It seemed of interest, therefore, to reinvestigate this question in order to deter-

mine: (a) the frequency with which preexisting valvular damage is found in cases of bacterial endocarditis; (b) whether or not activity of the rheumatic process is a necessary precursor to the superimposition of bacterial endocarditis; and (c) the mechanism or mechanisms that lead to the implantation of a bacterial endocarditis and, in particular, to the rôle rheumatic fever may play in such mechanisms.

### MATERIAL AND METHODS

As Libman and Thayer have emphasized, the division of the bacterial endocarditides into the acute and subacute forms based on the duration of the illness is largely arbitrary, inasmuch as on the one hand the clinical course may be prolonged for a number of months in frank cases of bacterial endocarditis which are known generally to run a very acute course (*e.g.*, *Staphylococcus aureus*), and on the other hand death may supervene within a few weeks in such usually protracted conditions as *Streptococcus viridans* endocarditis. In the latter, the shortening of the clinical duration is frequently due to fatal complications. Apart from this, it is often difficult to obtain a precise clinical history of the duration of the clinical phenomena so that one can never be certain that the course alleged to be under 6 weeks may not actually be longer. In spite of these difficulties we have deemed it best to classify our material into the subacute and acute groups for reasons that will become clear.

Subacute bacterial endocarditis, as is well known, is generally associated with organisms of low grade virulence and minimal pyogenic properties, and the duration of the illness is generally longer than 6 weeks. In the 42 unselected cases comprising this first group there was no history of primary bacterial infection elsewhere in the body. Included in this group were 6 cases in which the clinical history was shorter than 6 weeks. In practically all other respects, however, clinically as well as pathologically, these cases could not be distinguished from those in which the duration of the illness was more prolonged. The organisms recovered from the blood stream in these 6 cases were *Str. viridans* in 3, and gonococcus, pneumococcus and anhemolytic streptococcus (gamma) respectively in each of the remaining 3.



In the 28 unselected cases of acute bacterial endocarditis that comprise the second group, the course was very short (average 2 weeks), there were evidences clinically or pathologically of metastatic suppuration, and there existed a definite primary suppurating focus elsewhere in the body. The organisms recovered from the blood stream of these cases were definitely pyogenic, such as *Staph. aureus*, *Str. haemolyticus*, and so on.

Each group of cases was further subdivided, according to the criteria that are to be described, into "superimposed" cases, *i.e.* those that are implanted on a rheumatic process, and "non-superimposed" cases, *i.e.* those that do not appear to require primary rheumatic damage of the cardiac structures for their implantation.

The clinical histories, bacteriological findings, Wassermann test, electrocardiographic studies and pathological findings in the hearts and kidneys were carefully investigated. The hearts were fixed in 10 per cent formalin-saline \* and blocks were cut according to the standardized technique of Gross, Antopol and Sacks.<sup>52</sup> The staining methods were those employed by Gross and Ehrlich.<sup>42</sup>

### MACROSCOPIC FEATURES

The macroscopic features of subacute bacterial endocarditis have been clearly described by Harbitz,<sup>53</sup> Litten,<sup>54</sup> Libman,<sup>11, 13</sup> and Orth.<sup>55</sup> The vegetations presented extreme variations in their shape and consistence. In almost all instances they seemed to arise primarily on the closure line of the valves but were attached by a very broad base. In most of the cases the vegetations were fungating and friable. They often spread down the chordae tendineae of the mitral valve, causing rupture of these structures. In some instances the vegetations were flat and consisted of widespread granular irregularities. The color of the vegetations, as described by Libman,<sup>13</sup> may be yellowish, greenish, pinkish or reddish gray, depending somewhat on the organism and the duration of the process.

An important gross feature of the valvular lesion was its bilaterality, *i.e.* the tendency to affect both sides of the leaflet. This is due to the fact that the bacterial lesion frequently spreads around the

\* Solution of formaldehyde U.S.P., 10 parts; 1 per cent sodium chloride solution, 90 parts. This solution is rendered neutral with a weak alkali.

tip of the valve from the proximal layer<sup>50</sup> and produces a contiguity infection of the distal layer.

In one heart the center of the anterior mitral leaflet was ballooned out into an aneurysmal sac (Libman<sup>13</sup>) about the dimensions of a small filbert. This sac protruded toward the left auricular cavity. In another heart a similar aneurysmal bulging was present on the right aortic cusp. The aneurysm pointed toward the ventricular cavity and was in part ruptured and eroded.

Five hearts in which the aortic valve was chiefly or exclusively involved showed remarkable burrowing of the lesion into the subjacent myocardium of the interventricular septum or through the septum fibrosum. In 1 of these cases the septum fibrosum showed an aneurysmal bulging toward the right ventricle. In 3 of the cases the burrowing process had ruptured into the pericardial space between the aortic and pulmonic roots, producing a hemorrhagic and fibrinous contiguity pericarditis. In the 5th case\* the burrowing process had completely healed and the considerably deepened sinus of Valsalva disclosed a calcified finger-like projection from its base. The projection had apparently eroded the right cusp of the aortic valve. Two cases presented bicuspid aortic valves. The mechanism of their formation and their relation to rheumatic fever and subacute bacterial endocarditis has been reported elsewhere.

Large vegetations on the aortic cusps were almost invariably associated with a characteristic moth-eaten bacterial lesion on the ventricular surface of the anterior mitral leaflet. In most instances this was undoubtedly due to a contiguity infection from the aortic cusps. Such contiguity involvement of the mitral valves was observed in 14 of the 42 cases.

Of the mural endocardial lesions, the auricular bacterial process was the most characteristic and conspicuous. The vegetations presented the same appearance as on the valve. In the healing stages of the auricular bacterial endocarditis the vegetations were converted into small fibrous nodules producing a characteristic shark-skin appearance. Other significant mural lesions consisted

\* This case, which will be published *in extenso* by Averbuck and Libman, is one of undoubted subacute bacterial endocarditis which apparently underwent complete healing (with possible bacterial residua deep in the scar tissue). Eight years after the process was considered healed the patient developed a superimposed acute bacterial endocarditis (*Staphylococcus albus*) from which he died.

of: (a) generally small vegetations which spread down the chordae tendineae to implant at the apices of the papillary muscles in the left ventricle; (b) contiguity spread of the bacterial process below the base of the aortic valve, involving the left side of the inter-ventricular septum for a variable distance (Libman<sup>11</sup>); and (c) spread of the lesions up the sinus of Valsalva to involve the aortic root. A characteristic lesion seen in instances of septum fibrosum perforation (not included in this group) is impact implantation of bacterial vegetations on the tricuspid valve or right ventricular wall distal to the septum. The location of such lesions definitely indicates that a fine spray of infected blood from the systolic discharge of the left ventricle initiates the mural vegetative bacterial process. Other mural lesions were not found in the cases comprising this group.

Although the gross appearance of the bacterial lesions in the "superimposed" subgroup was similar to that in the "non-superimposed," the former subgroup could be generally identified by the presence of the macroscopic rheumatic lesions which have been fully described elsewhere. Of considerable aid in determining previous underlying rheumatic damage are the following features: thickening and deformity of the valve at sites not in the immediate vicinity of the bacterial process; thickening and absorption of the chordae tendineae; gross vascularization of the valve (very important); straightening of the free edges of the auriculoventricular valves; aortic commissural fusion; notching of the aortic cusps; very delicate tooth-like prominences of the pulmonic valve free edges; old flat rheumatic left auricular lesions; and pericardial adhesions behind the left auricle. It is of interest to note that 3 of the cases in this group showed advanced button-hole mitral stenosis.

In the non-superimposed group, valve vascularization was never seen grossly; the only gross left auricular endocardial lesions present were those obviously due to the bacterial process, and the portions of the valve leaflets not in the immediate vicinity of the bacterial vegetations were delicate and translucent.

As noted by Harbitz<sup>53</sup> and by Libman,<sup>11</sup> macroscopic indications of healing are not infrequently conspicuous in this condition. This is evidenced by the formation of mounds and ridges of scar tissue around the bacterial process and by the conversion of the

vegetations into irregular, often large, sharply etched, calcific masses not infrequently projecting for a considerable distance beyond the confines of the valve leaflets. The contour of these calcified vegetations was usually distinguishable from secondary calcific changes in rheumatic valves, the latter generally conforming to the topography of the deformed leaflets and rarely projecting an appreciable distance from their surface. They could furthermore be differentiated from the Mönckeberg calcific aortic process<sup>56</sup> in that this latter generally produces more or less smooth nodular excrescences within the cusps and fused commissures, which also remain relatively confined to the valvular tissue.

In several instances fine granular verrucae occupying the characteristic position of the typical rheumatic variety were seen on the closure line chiefly of the mitral and aortic, though sometimes the tricuspid valve. Because of their small size, tawny color, firm consistence, delicacy of contour and distribution on the closure line, one could suspect an associated fresh rheumatic lesion in such cases. This has also been previously observed by Libman.<sup>17</sup> It must be stated, however, that in the absence of these characteristics it does not seem justifiable to make a diagnosis of an active rheumatic process. It will be shown that bacterial vegetations may also exist in the form of small verrucae and furthermore that small non-bacterial vegetations occur in the proximity of bacterial masses even in cases in which there is no evidence of rheumatic infection, past or present.

In 81 per cent of the 42 cases, bacterial vegetations were present on the mitral valve, and in 66 per cent on the aortic valve. In 43 per cent of the cases the mitral and aortic valves were involved together, with or without associated lesions on other valves and left auricular endocardium. The mitral valve, or mitral valve and left auricle, was involved as the only bacterial process in 30 per cent of the cases, and in 25 per cent the aortic valve alone was involved. The tricuspid valve was involved in 6 cases. In 1 case with negative blood cultures and a large interauricular septal defect, the tricuspid valve was the only one affected by the bacterial lesion. In the remaining 5 cases *Str. viridans* was recovered from 3, *Str. haemolyticus* from 1, and the blood cultures were negative in 1. The pulmonic valve (and all the other valves) was in-

volved in 1 case with *Str. viridans* infection. The left auricle was involved in 26 per cent of the cases.

From the entire group of 42 cases, negative blood cultures were obtained in 10, *Str. viridans* in 26, gonococcus in 4, influenza bacillus in 3, and hemolytic streptococcus, anhemolytic streptococcus (gamma) and pneumococcus, each in 1 case. It is of interest to note, in view of the observations by previous workers concerning the predilection for the gonococcus to lodge in the right heart, that this was not observed in our cases. Apart from the fact that the duration of the illness was usually shorter in the cases with gonococcus, pneumococcus and anhemolytic streptococcus (gamma) infection, there were no appreciable differences between these and those due to *Str. viridans*.

Because of the limited number of cases on which these studies have been made, it is hazardous to come to definite conclusions concerning differences in localization of the bacterial endocarditic process as between the superimposed and non-superimposed subgroups. It appears, however, that left auricular involvement and the combination of mitral and aortic lesions was more frequent in the superimposed cases. On the other hand, involvement limited to the aortic valve alone was found more frequently in the non-superimposed subgroup. Apart from this there is a decided similarity between the superimposed and non-superimposed cases as regards the nature, extent and distribution of the bacterial vegetations. It has already been indicated that differentiation between these groups can be made by the associated valvular and other endocardial lesions. In most instances this differentiation can be determined with great accuracy by the microscopic features to be described.

### MICROSCOPIC FINDINGS

The most conspicuous histological feature of the valvular lesion in subacute bacterial endocarditis, irrespective of whether it was superimposed on a rheumatic process or non-superimposed, consisted of irregular vegetations which were generally of considerable size (Figs. 1, 6, 7, 8). In approximately half of the cases bacteria could be seen within the superficial as well as deep layers of the vegetations. In about one-fifth of the cases the bacteria

appeared to be largely superficially situated, and in the remaining cases they were generally deep. Apart from the bacteria, the vegetations themselves usually appeared to consist largely of platelet material sometimes admixed with fibrin. In about one-third of the cases the platelet material took a peculiar form which somewhat resembled necrotic liver tissue. It is of importance that, in contrast with the acute bacterial vegetations to be described later, polymorphonuclear leukocytes were generally absent from these vegetations and the bacteria often appeared in the form of clumps or packets.

Verrucae which did not disclose bacteria were noted side by side with the bacterial vegetations and occasionally not in their immediate vicinity. In the superimposed cases some of the verrucae appeared to be of rheumatic origin (Fig. 2). However, the fact that somewhat similar verrucae were also observed in the non-superimposed cases in which there was no evidence either in the form of rheumatic stigmata or in the presence of Aschoff bodies that there was present an underlying rheumatic lesion, indicates clearly that one is not justified in accepting such verrucae as evidence of an active rheumatic process. Apparently, then, as a result of the toxic irritative effects of the bacterial infection, non-bacterial verrucae may form on the valves. Their structure may be somewhat similar to rheumatic vegetations.

A destructive process was generally present in the valve. Occasionally this was severe, at other times minimal. Associated with the destructive process there were often present lymphocytes and polymorphonuclear leukocytes, sometimes large mononuclear cells. In approximately 25 per cent of the cases the base of the vegetations showed a reactive process in the form of irregularly distributed large polygonal cells with sharply outlined basophilic protoplasm and fibrocytoid nuclei. At times these cells were multinucleated. It was difficult to determine whether they represent proliferated fibroblasts, macrophages or endothelial cells. We are inclined to agree with Jaffé<sup>57</sup> that the fibroblast is the chief source of these reactive cells. Their number, shape and distribution appeared to occupy an intermediate position between those seen in the rheumatic process and those in the acute endocarditides. In the former, somewhat similar cells are often seen in considerably greater numbers at the base of the verrucae (Fig. 3). On the

other hand, in the 28 cases of acute bacterial endocarditis to be described, these cells were very infrequent and when present showed pyknotic nuclei with rather aborted cytoplasm.

The valvular lesion essentially due to the bacterial process was most clearly seen in the non-superimposed cases. Vascularization was confined to the formation of capillaries. These were often present in the vicinity of a moderately active inflammatory lesion of the valve. In the neighborhood of vegetations, capillaries were sometimes present in extraordinarily large numbers (hypercapillarization) and many were seen to communicate directly with the cardiac chambers. The remainder of the valve leaflet not infrequently showed a scattering of lymphocytes and large mononuclear cells.

In the superimposed cases vascularization with muscular vessels was invariably noted in the mitral valve (Fig. 2) and frequently in the others. Together with the larger vessels, one could generally observe a sheaf of arterioles and capillaries which were obviously a continuation from the ring region. With few exceptions such valves presented fibroelastic reduplication of the proximal valve layers and valve angles, which were often vascularized, formation of collagenous thickening of the valve tip with dipping of the elastic lamellae beneath the scarred mass, reduplication of the endocardial layer of the chordae tendineae with absorption, and widening of the vascularized spongiosa layer. In a few cases evidence of previous rheumatic damage was confined to the presence of a few thick walled vessels within the auricularis layer of the auriculoventricular valves. In such instances, however, there were present elsewhere in the heart other widespread, even though at times inconspicuous, stigmata of an ancient rheumatic infection.

One of the most interesting and significant lesions, which, in its typical form, is apparently confined to subacute bacterial endocarditis, is what we have termed the "spongy" lesion (Figs. 4, 5). This consists of a collection of wide anastomosing cavernous channels presumably originating from dilatation of capillaries in a hypercapillarized area. On serial section these vascular channels were seen at times to communicate directly with the cardiac cavity as well as with other valvular vessels. Although a process somewhat resembling this spongy transformation was noted on rare occasions in very active rheumatic valvular lesions and, as will be

described, in 1 case of acute bacterial endocarditis, the histological appearance of the lesion in those cases differed considerably from the typical large mass of cavernous channels found in subacute bacterial endocarditis. In the rheumatic process, as well as in the case of acute bacterial endocarditis, the spongy lesion was represented by an aborted attempt at such cavernous transformation. Spongy lesions were present in 50 per cent of the cases of subacute bacterial endocarditis. Their site of predilection was in the vicinity of healing vegetations, near chordae tendineae insertions, and in the valve pockets.

When the bacterial process involved the aortic valve to any considerable degree, the inflammatory process was frequently observed spreading through the aortic annulus (Figs. 6, 7), producing at times a contiguity infection of the adjacent pericardium. Because of the considerable necrosis, which occurs in some of the cases, it becomes clear that such a contiguity process may lead to rupture behind or through the sinus of Valsalva with severe infection of the pericardial mantle. This occurred in 3 of the cases and has some clinical significance.

Not infrequently the valve leaflets not directly involved by the bacterial process presented a varying degree of infiltration with mononuclear cells. Although such a mild interstitial valvulitis may be expected to occur in the leaflets of the superimposed cases because of the underlying rheumatic valvulitis, its occurrence in the non-superimposed cases suggests that the infiltration is the result of a toxic irritative process.

When bacterial vegetations did not encroach on the valve rings in the non-superimposed cases, this site was invariably free of newly formed capillaries. In the aortic and mitral rings which normally possess no vasculature (except for occasional isolated capillaries in very few instances), capillarization was not observed in the non-superimposed cases. Similarly, in such instances the ring region was free from inflammatory cells. On the other hand, in the superimposed cases, *i.e.* those in which there was an underlying rheumatic basis, almost all rings disclosed vascularization, capillarization and scattered lymphocytes. Obviously, therefore, this was a valuable index of such previous rheumatic change. However, in the presence of bacterial vegetations in the proximity of the rings, capillarization and sometimes hypercapillarization



was frequent. Together with these, there was also present other cellular manifestations of acute inflammatory involvement.

Subacute bacterial endocarditis *per se* was not infrequently associated with a mild scattering of mononuclear cells in the endocardium of the left auricle. As with the ring lesion, the non-superimposed cases were conspicuous for their absence of capillary penetration and the various endocardial reduplications characteristic of the rheumatic process. Furthermore, in the immediate vicinity of bacterial vegetations there occurred a very florid formation of capillaries which penetrated the endocardium in large numbers to reach the base of the vegetations. This process differs very decidedly from the rheumatic endocardial lesion in which capillary penetration is at a minimum and in which other phenomena such as palisading with basophilic mononuclear cells, and eosinophilic necrosis and swelling of the collagen, are not infrequently present. In superimposed cases with active rheumatic lesions such auricular endocardial inflammatory processes of both types may be seen side by side.

In most instances of subacute bacterial endocarditis the myocardium throughout the heart showed scars in various stages of healing. Apart from this, there was frequently present mild interstitial mononuclear infiltration which was occasionally focal (so-called Bracht-Wachter lesions). Polymorphonuclear leukocytic interstitial myocarditis was rare. Aschoff bodies were found in 9 of the 42 cases (21 per cent). They were all present in the 30 superimposed cases, an incidence of 30 per cent. The significance of this observation will be taken up in the discussion. The blood vessels in the myocardium, particularly the posterior papillary muscle, posterior wall of the left ventricle and interventricular septum, frequently presented fibroelastic alterations. In a few instances thrombosed vessels were noted in the myocardium. In 1 case there was also present a necrotizing arteritis. No bacteria were demonstrable in these lesions.

The horizontal conduction system (bundle of His) presented scattered mononuclear cells and lymphocytes in 3 cases. In 6 additional cases the septum fibrosum showed focal collections of lymphocytes, mononuclear cells and occasionally polymorphonuclear leukocytes with capillarization and the production of muscular walled vessels. In 3 cases the septum fibrosum was acutely

inflamed and necrotic, with abscess formation in 1 instance. It is of interest to note that these lesions of the bundle and septum fibrosum were found only in the superimposed cases.<sup>47</sup>

Similarly, whereas no alterations were encountered in the intervalvular fibrosa or roots of the great vessels in the non-superimposed cases, except in the proximity of a bacterial process, capillarization with scatterings of lymphocytes was observed in the intervalvular fibrosa in approximately one-third of the superimposed cases, and in the great vessel roots in over one-half of the cases. In 1 instance the aortic root showed destruction of the elastica with abscess formation and contiguity spread to the pericardium.

The pericardium showed evidences of irritation in practically every case. The alterations consisted of scattered large mononuclear cells, less frequently lymphocytes and polymorphonuclear leukocytes, thickening of the septa between the fat cells, thickening and hyalinization of the precapillary and arteriolar walls with, occasionally, necrobiotic changes in these, and in a few instances thrombosis. There were absent, however, the characteristic proliferated capillaries, peculiar large bodied mononuclear and binucleated cells and the signet formations seen in Libman-Sacks disease.<sup>58</sup> Moreover, the vascular alterations were not as frequent or as intense as in that condition. There was also absent the tendency for lymphocytes to localize around the lamina propria, and for the development of corkscrew capillaries and larger vessel changes characteristically present in the rheumatic pericardial lesions.<sup>48</sup>

Finally, apart from the lesions described above, 8 of the cases with a duration of 1 year or longer presented certain histological peculiarities. Evidence of healing was very considerable. In several of these cases bacteria were found only with difficulty. They were indistinct in outline, poorly staining and were generally deeply embedded in relative acellular collagenous tissue (Fig. 8). All but 2 of these cases failed to disclose bacteria on culture. Four of these had been diagnosed as in the bacteria-free stage according to Libman's criteria,<sup>11, 13, 14, 16, 17</sup> and 1 was considered healed. In the 2 cases with positive blood cultures the organism recovered was *Str. viridans*. In view of the discussion, which will be taken up subsequently, it is interesting to note that

3 of these 8 cases showed typical Löhlein lesions in the kidneys. One case presented a chronic diffuse nephritis. Six of the 8 cases were superimposed on an old rheumatic valvulitis.

In summary, the 42 cases of subacute bacterial endocarditis could be divided macroscopically, but with greater certainty microscopically, into 30 cases which were superimposed on a rheumatic basis and 12 cases in which there was no underlying rheumatic alteration of the valves. The gross features by which this differentiation into the "superimposed" and "non-superimposed" subgroups can be made have already been described. As regards the distribution of the bacterial vegetations, it need only be noted that left auricular endocardial involvement and the combination of mitral and aortic involvement appeared to be more frequent in the former subgroup, whereas bacterial vegetations confined to the aortic valve were more frequent in the latter. Histologically, the superimposed group could be identified by the presence of the characteristically rheumatic left auricular endocardial and valvular reduplications, valve angle lesions, vascularization and capillarization of the valve rings, leaflets, intervalvular fibrosa, bundle, septum fibrosum and great vessel roots, and by the presence of Aschoff bodies in 30 per cent of the cases. No Aschoff bodies were found in the non-superimposed subgroup. Furthermore, capillarization and inflammatory cell infiltration were absent except in the proximity of a bacterial process. Non-bacterial vegetations were found in both superimposed and non-superimposed cases. Apart from these, the lesions produced by the subacute bacterial endocarditis were as follows: deposition of vegetations which were generally irregular, often large in size, frequently showing blood platelet structure resembling necrobiotic liver tissue and containing, either superficially or deeply, packets of bacteria; reaction at the base of these vegetations in approximately one-fourth of the cases, in the form of peculiar, large, irregularly polygonal mononuclear and sometimes giant cells with basophilic protoplasm; capillarization or hypercapillarization in the proximity of the vegetations; spongy lesions in approximately one-half of the cases; a varying degree of necrosis of the valve leaflet; and the bilaterality of these lesions.

## ACUTE BACTERIAL ENDOCARDITIS

Of the 28 non-selected cases falling in this group, 21 were superimposed on a previous rheumatic process and 7 were non-superimposed. Although this proportion of cases in which there was underlying rheumatic damage is possibly somewhat higher than would be disclosed by more extensive material, it appears that acute bacterial endocarditis does not differ essentially from the subacute form in respect to the important rôle that previous rheumatic damage plays in predisposing the valve to a bacterial process, a point to which little attention has been paid.

So far as the gross appearance of these vegetations is concerned, there is often little to differentiate them from the subacute bacterial endocarditis. On the whole, however, the vegetations tend to be considerably smaller, evidence of healing is generally absent, and destruction of valve tissue may be considerable. The distribution of the bacterial vegetations in these 28 cases was as follows: mitral valve 20 cases; aortic valve 13 cases; combination of mitral and aortic valve 7 cases; tricuspid valve 4 cases; pulmonic valve 1 case; and left auricular endocardium 2 cases. This distribution is strikingly similar to that observed in subacute bacterial endocarditis except for the considerably lower incidence of left auricular involvement. No appreciable differences were observed between the superimposed and non-superimposed subgroups.

The histological differentiation of the acute from the subacute bacterial endocarditis was rendered relatively simple in most instances. Thus, in the acute bacterial cases the vegetations were generally small and flat; the curious liver-like formation was observed in only 2 cases; the vegetations usually consisted of almost solid bacterial masses (Figs. 9, 10), or fibrinous material through which the bacteria were diffusely distributed (not in packets as in subacute bacterial endocarditis), almost invariably reaching the surface of the lesions and often permeated by necrobiotic polymorphonuclear leukocytes; in a number of instances the bacteria could be seen invading the interstices of the collagenous structure of the valve; the underlying valvular tissue showed extreme necrosis and suppuration (Fig. 9), generally far greater than that seen in the subacute group; cellular reaction immediately beneath the bacterial masses was minimal; large mononuclear cells with

somewhat scantier protoplasm than that seen in subacute bacterial endocarditis and with pyknotic nuclei were seen at the base of these bacterial lesions in only 2 instances; capillarization in the proximity of the bacterial vegetations was often minimal and the spongy lesion, in considerably aborted form, was seen in only 1 case. This latter was the most valuable differentiating feature, as was the presence of abscesses in the myocardium.

As in the subacute group, the characteristic rheumatic stigmata readily differentiated the superimposed from the non-superimposed groups (Fig. 10). Thus, the non-superimposed cases of acute bacterial endocarditis were not associated with alterations of the valve ring, intervalvular fibrosa, great vessel roots or left auricular endocardium except in the immediate proximity of a bacterial vegetation. Irritation processes were seen in the pericardium in these as well as in the superimposed cases. Except for a greater tendency to polymorphonuclear leukocytic reaction it was difficult to differentiate the irritative phenomena in the pericardium of this group from the subacute. Aschoff bodies were found in 6 of the 21 superimposed cases (29 per cent).

In summary, acute bacterial endocarditis produces a rapidly destructive valvular lesion, capillarization is at a minimum, vascularization with muscular vessels is absent (except in the superimposed cases), the bacteria are superficially placed on the valvular substance, platelet formation is infrequent, vegetations consist of large solid masses of bacteria and diffuse infiltration with polymorphonuclear leukocytes is not infrequent. The typical spongy lesion was not present in these cases, thus forming a very characteristic differentiating feature. The distribution of bacterial vegetations was somewhat similar to that found in subacute bacterial endocarditis. Ring lesions, as well as other rheumatic stigmas were absent except in the superimposed cases where they could be definitely attributed to the underlying rheumatic process. Myocardial abscesses were frequent in cases of acute, and rare in subacute bacterial endocarditis.

## DISCUSSION

It was indicated in the introductory remarks that the duration of the illness and the organism isolated from blood culture are

useful, but by no means certain, methods for differentiating acute from subacute bacterial endocarditis. Certain clinical features, particularly those emphasized by Libman, are of considerable value in attempting to make this distinction. Thus, for example, it has been observed that a bacterial endocarditis in the presence of a primary suppurative process elsewhere in the body generally indicates the acute form of the disease, whereas the absence of such a primary lesion in the presence of hematuria (Harbitz,<sup>53</sup> Libman<sup>11</sup>) is strongly indicative of the subacute form.

A fundamental contribution to the pathological lesions of the kidneys responsible for the hematuria was made by Löhlein<sup>59</sup> in 1910. This observer reported that in chronic "ulcerative endocarditis" there is occasionally found in the kidneys what he considered to be an embolic focal glomerulonephritis. Löhlein's article contains an excellent description and beautiful illustrations of this lesion. These observations were subsequently confirmed by Baehr,<sup>60, 61</sup> and by Gaskell.<sup>62</sup> The former, studying the kidneys from Libman's large collection, noted 1 example of acute diffuse glomerulonephritis, 1 of chronic diffuse glomerulonephritis, and 84 of Löhlein lesions among 91 cases of subacute anhemolytic streptococcus endocarditis with positive blood cultures. Chronic diffuse glomerulonephritis was present in 11 of 34 cases in the bacteria-free stage, and 19 cases showed Löhlein lesions. Baehr shared with Löhlein the belief that the focal glomerular lesions are due to bacterial emboli. In 1932 Bell<sup>63</sup> stated that he found Löhlein lesions in over 50 per cent of his 108 cases of subacute bacterial endocarditis and in 8 out of 125 cases of acute bacterial endocarditis.

In an examination of the kidneys from our cases we have observed the Löhlein lesion in approximately half the cases of subacute bacterial endocarditis. As already mentioned, 1 case with negative blood cultures showed a chronic diffuse glomerulonephritis and 1 case with gonococcus endocarditis presented an acute diffuse glomerulonephritis. Eleven of the cases with negative kidneys were associated with a *Str. viridans* endocarditis. On the other hand, Löhlein lesions were present in 1 case of subacute bacterial endocarditis due to hemolytic streptococcus, in 3 cases due to influenza bacillus, and in 2 cases due to gonococcus. Although most of our cases of acute bacterial endocarditis showed glomerular altera-

tions chiefly in the form of thickening of the capillary loops, in only 2 cases were there found changes which were somewhat similar to the Löhlein lesion. Study of the pathogenesis of these focal glomerular alterations has led us to the conclusion that they represent a focal toxic proliferative and necrotic process, with possibly also thrombus formation. We do not favor the view that the changes are brought about by bacterial embolization. In this respect, therefore, the pathogenesis of this lesion is similar to the so-called Osler node which is considered by Lian, Nicolau and Poincloux,<sup>64</sup> and by Merklen and Wolf<sup>65</sup> to result chiefly from a proliferative endothelial process. The infrequency of Löhlein lesions in acute bacterial endocarditis is possibly due to the short duration of the illness and also to the fact that the bacterial toxins in these acute cases are profoundly necrotizing and lack the ability to stimulate proliferation of the endothelial cells. The milder stimulating properties of the toxic principles from the organisms responsible for subacute bacterial endocarditis (usually *Str. viridans*) lead to a more protracted and less stormy clinical course, to proliferation of subcutaneous capillary endothelium (Osler nodes) and glomerular capillary endothelium (Löhlein lesions) and to the proliferative phenomena in the valves.

Whatever the pathogenesis of the Löhlein lesion may be, its occurrence in acute bacterial endocarditis, and rarely in cases without endocarditis, as well as the finding of diffuse glomerulonephritis in subacute bacterial endocarditis, renders this focal renal lesion a useful but not an absolute means for differentiating these conditions.

In the descriptions given above we have indicated that the histological appearance of the valvular and mural lesions is a valuable aid in distinguishing acute from subacute bacterial endocarditis. We have further shown that it is possible to subdivide the endocarditides into those that are superimposed on a rheumatic process and those that appear to have been deposited on non-rheumatic valves. It is important to bear in mind that in most instances this subdivision can be made with considerable certainty since the superimposed cases present a widespread distribution of unmistakable rheumatic stigmata. It is, of course, conceivable that rheumatic fever may heal with complete restitution to integrity of the tissues without leaving discernible traces.

With our present knowledge, however, we have no means for recognizing such completely effaced lesions, granted this takes place. With this reservation in mind, therefore, we must conclude that in 25 per cent of our cases of both subacute and acute bacterial endocarditis, no underlying rheumatic alteration was present in the valves. In 1 case of subacute bacterial endocarditis there was a co-existing syphilitic lesion of the aortic valve, and in another a calcific aortic sclerosis of the Mönckeberg type. Two cases were associated with bicuspid aortic valves; these were, however, on a rheumatic basis.<sup>34</sup> This still leaves an appreciable proportion of cases without any discernible valvular alterations.

We have already stated that small non-bacterial verrucae are at times found side by side with bacterial vegetations in both superimposed and non-superimposed bacterial endocarditis (Fig. 10). In a few of the superimposed cases these verrucae occupied the typical closure line position and presented the characteristic structure of rheumatic verrucae. In all of these cases the presence of Aschoff bodies in the myocardium confirmed our belief that these verrucae represented a simultaneous active rheumatic infection. In a few cases Aschoff bodies were found in the myocardium but no typical rheumatic verrucae were noted on the valves. In appraising the histological appearance of the verrucae, one is aided by the fact that the typical rheumatic verrucous mass is not of platelet structure; there is a great tendency to its invasion by granulation tissue, chiefly fibroblasts; and the lesions are generally close cropped. In the more active cases, especially in younger persons, the base of the rheumatic verrucae frequently presents large mononuclear cells somewhat similar to those seen in the subacute process except for their considerably greater numbers. Verrucae possessing all these characteristics were found in a minority of the superimposed cases and in none of the non-superimposed cases. In both these groups there were observed small non-bacterial verrucae which, in our opinion, could not be accepted as indicative of an active rheumatic process inasmuch as they lacked many of the characteristics described above, but chiefly because they also occurred in hearts that presented no other stigmata of rheumatic fever. Since the incidence of the more typical rheumatic verrucae was lower than that of Aschoff bodies in the myocardium, and since the Aschoff bodies were present in only 30 per cent of the



superimposed cases, we cannot agree with VonGlahn and Pappenheimer's conclusion that infection of cardiac valves with *Str. viridans* in rheumatic patients is due to the implantation of bacteria on unhealed rheumatic verrucae.

As was previously indicated, Aschoff bodies were found in 45 per cent of Clawson's series of 60 cases, and in 46 per cent of VonGlahn and Pappenheimer's 26 cases. In our own material, Aschoff bodies were present in 9 of the 30 cases of superimposed bacterial endocarditis (30 per cent), in none of the non-superimposed cases, and in 6 of the 21 cases of superimposed acute bacterial endocarditis (29 per cent). Inasmuch as Gross, Antopol and Sacks<sup>52</sup> found Aschoff bodies in only 15 per cent of chronic rheumatic cases, it appears that the higher incidence of these lesions in the bacterial endocarditides is of some significance. For reasons which we have stated above, we cannot agree with VonGlahn and Pappenheimer's view as to the invariable presence of rheumatic activity in these hearts, and therefore believe that if subacute bacterial endocarditis of the non-hemolytic streptococcus type is superimposed on unhealed rheumatic vegetations, this occurs in only a minority of the cases. A further difficulty to the acceptance of their theory is the fact that rheumatic children whose hearts are much more frequently the seat of activity and whose valves also show a much greater frequency of fresh verrucae, are far less apt to develop subacute bacterial endocarditis than are the hearts of adults in whom the chronic rheumatic process is much more frequently found. While it is true that other factors may play a rôle to explain this discrepancy, for example, it may be argued that intercurrent infection may be less frequent in childhood than in the later age periods, there is no definite evidence to support this view. On the contrary, in a study of blood invasion in a variety of conditions, carried out by Lichtman and Gross,<sup>66</sup> it has been shown that streptococcic invasion occurs in approximately 6 per cent of cases. The age of the patient did not appear to play a rôle. Unless some other factor is involved inimical to the formation of bacterial endocarditis in childhood, it would appear that it is the chronic rheumatic valve with its peculiar aptitude to the formation of eosinophilic necrosis of collagen which is more likely to become the seat of a bacterial endocarditis than is the fresh vegetative lesion.

It is well known that a variety of infectious diseases may eventually precipitate an acute attack in an otherwise inactive rheumatic process (Libman <sup>17</sup>). It may well be, therefore, that an implantation of a bacterial endocarditis in such cases may similarly lead to reactivation of a smoldering rheumatic infection. This view, which has been recently suggested by Graybiel and White,<sup>67</sup> appears to be consistent with the facts and affords an explanation for the higher incidence of Aschoff bodies in the superimposed bacterial endocarditides.

There remains to be considered the possible mechanisms that lead to the implantation of a bacterial endocarditis. The great frequency with which underlying rheumatic valvular damage plays a rôle is clear indication of a causal connection between the two, even though, as has been indicated, the presence of fresh verrucae apparently does not adequately account for the susceptibility of the valvular structure. Since the announcement by Koester <sup>68</sup> of the embolic theory of endocarditis 59 years ago, many investigators have assumed that the valvular lesions in bacterial endocarditis were due to the lodgement of bacteria in the vessels of the cardiac valves. The generally rich vascularization of rheumatic valves was considered to afford a plausible explanation for the predisposition of such altered valve leaflets to the implantation (by embolization) of a bacterial endocarditis. Grant, Wood and Jones <sup>69</sup> have stated their reasons for not favoring this embolic theory. We are in full agreement with these authors for the following reasons:

1. In spite of the fact that in rheumatic valvulitis the valve rings are much more frequently vascularized than the valve leaflets, a superimposed bacterial endocarditis occurs frequently on the closure line of the leaflet and rarely at the ring.
2. If bacteria were to lodge in vessels within the valve they would produce an interstitial valvulitis. However, the bacterial endocarditides are not primarily associated with an interstitial valvulitis but appear to arise by implantation on the surface of the leaflets.
3. Furthermore, in true interstitial valvulitis (*e.g.* in rheumatic fever) the lesion is always observed to spread from the ring toward the valve tip, whereas in the bacterial endocarditides the rings are not involved primarily.

4. Twenty-five per cent of our cases were not superimposed on rheumatic valves, and since normal valves do not possess blood vessels,<sup>70</sup> another explanation must be sought to explain this discrepancy.

Furthermore, in considering the remarkable manner in which the incidence of both the acute and subacute bacterial valvular lesions in superimposed as well as non-superimposed cases parallels that of the rheumatic valvular lesions, and also the fact that congenital and acquired cardiac anomalies are well known for their susceptibility to bacterial endocarditis, it seems to us that at least two mechanisms may play a rôle in this connection, *viz.* intrinsic changes in the endocardial tissues (rheumatic deformity, congenital defects) which transform them into a more suitable anchoring ground for transient bacterial invaders; and hydrodynamic factors (tension) which favor implantation of bacteria and aid in the progress of the infection.

As regards the hydrodynamic factors, it has already been shown by Gross and Friedberg<sup>50</sup> that the formation of rheumatic verrucae is considerably influenced by mechanical stress and strain. Thus, it was pointed out that the closure lines of those valve leaflets that are subjected to the greatest pressure changes (left heart) become the seat of verrucous formation. In valve leaflets that have become so stiffened and deformed that they cannot close, a new line of verrucae forms on the site most exposed to eddies and blood currents. Moreover, while the incidence of rheumatic verrucae is usually highest in the left heart, it may become significantly increased in the right heart under conditions that produce increased tension in the lesser circulation. Recent experimental observations by Nedzel<sup>71</sup> have convinced him that "pressor episodes" may induce valvular preparation (adhesiveness) as well as a bacteriemia.

An examination of the data submitted above, concerning the localization of bacterial endocarditis, reveals the following pertinent facts:

1. Bacterial verrucae are apt to localize with preference on the closure line or on the most projecting portions of the valves.
2. In a case of patent foramen ovale with increased tension in the right auricle, the only bacterial valvular lesion found was on the tricuspid leaflet.

3. In cases with perforated septum fibrosum, bacterial vegetations frequently form on the mural endocardium of the right heart opposite the stream of blood ejected from the left heart during systole.

4. The aortic sinuses, which are exposed to considerable tension both during systole and diastole, are apt to show conspicuous progress of the bacterial process, with undermining of the tissues and in some cases rupture into the retroaortic pericardial space.

5. Bacterial endocarditis is apt to occur in the proximity of such congenital cardiac defects as are associated with alterations in the direction or intensity of the blood currents. The endocardium surrounding these defects frequently shows proliferative changes.

These observations clearly indicate that the same hydrodynamic factors which are operative in determining the localization of rheumatic verrucae also serve to anchor transient invading bacteria and to promote the progress of the infection. It is of interest in this connection that these sites of increased tension almost invariably show proliferative changes. It appears possible, therefore, that such proliferative changes either by themselves or because of secondary local intrinsic changes, such as thrombosis (von Jürgensen <sup>72</sup>) or degeneration, afford optimum conditions for bacterial implantation.

Apropos of this latter (intrinsic changes) we are in full agreement with Grant, Wood and Jones <sup>69</sup> that these sites not infrequently present thrombotic lesions during life. Gross and Friedberg <sup>73</sup> have recently shown that the closure line of old rheumatic valves may become the seat of large thrombotic deposits. The pendulous stalks that are occasionally found on such deformed valves seem to indicate their origin from healed thrombotic vegetations. It appears, therefore, that lesions of this type are not necessarily terminal events. Furthermore, the closure line of deformed valves not infrequently presents eosinophilic necrosis of collagen.<sup>50</sup> This, too, may serve as a suitable anchoring ground for bacteria. Apart from rheumatic alterations and non-bacterial thrombotic deposits, proliferative changes in the valvular endothelium also occur in calcific sclerosis of the aortic valve (Mönckeberg type) and in aortic valve incompetency due to syphilis. In

the latter, proliferation of the endocardium is frequently observed on the septal aspect of the left outflow tract immediately below the aortic cusps. We have observed 1 case in which a bacterial endocarditis seemed to be engrafted on such a proliferated area. In addition to these, Friedberg and Gross<sup>58</sup> have shown that in a small per cent of a variety of conditions, including commonly encountered infectious processes, anemias, and so on, small proliferated lesions (eosinophilic multinucleated bodies) may be found on the surface of the valves. They occur more frequently on rheumatic valves than on normal ones. Finally, in a case studied clinically by Libman and reported by Gross,<sup>74</sup> subacute bacterial endocarditis was found to be engrafted on an atypical verrucous endocarditis (Libman-Sacks type). These observations collectively indicate that any process that leads to alterations of the valvular or mural endocardium (thrombotic, proliferative or necrotic) whether these be due to inflammatory changes (rheumatic fever, Libman-Sacks disease) to primarily degenerative processes (Mönckeberg sclerosis), mechanical alterations in the impingement of blood (lentic aortitis, congenital defects), or toxic principles (eosinophilic multinucleated bodies) transforms the local sites into a suitable anchoring ground for bacteria. We do not deny the possibility of implantation on fresh rheumatic verrucae, but believe that this is considerably less important than the factors mentioned above. Nor, indeed, do we believe that the mechanisms discussed are the only ones involved. With our present knowledge, however, it seems justifiable to assume that these play an important rôle even though there may be other contributing factors.

There remains to discuss briefly the possible mechanisms involved in the implantation of bacterial endocarditis in the non-superimposed cases. It has been shown that both non-bacterial thrombotic verrucae<sup>73</sup> and eosinophilic multinucleated bodies<sup>58</sup> also occur on non-rheumatic valves but less frequently than on the rheumatic ones. Furthermore, de Vecchi<sup>75</sup> and others have observed proliferative changes on the valve in a variety of conditions. We have then in this group some of the components in common with those found in rheumatic valves. However, the relative infrequency of these alterations, and the absence of eosinophilic collagen necrosis and other proliferative changes, may explain, at least in part, the lower incidence of bacterial endocarditis

in the non-rheumatic cases. Granted bacterial implantation takes place, the subsequent events will be largely determined by the nature of the organism. If this is of a type that produces a mild stimulating toxin and a prolonged course, proliferative changes will take place in the valve, subcutaneous and renal capillaries, and a smoldering rheumatic infection may be awakened into activity. If the invading organism produces toxins of a drastic necrotizing potency, the clinical course will be short, the local lesions (valves) will be suppurative and necrotic, and proliferation will be minimal. In these cases, however, the factors necessary for the stimulation of the Aschoff body reaction are still operative. It seems to us that a working hypothesis along these lines more adequately explains the similarities as well as differences between acute and subacute bacterial endocarditis.

### SUMMARY AND CONCLUSIONS

There have been described in this report the macroscopic and microscopic findings in the hearts in 42 cases of subacute bacterial endocarditis, and 28 cases of acute bacterial endocarditis. It is shown that while there is no sharp line of distinction between these conditions and a variety of lesions are common to both, certain clinical, bacteriological, anatomical and histological features are of considerable aid in classifying the bacterial endocarditides into these two categories. An important, differentiating histological feature is the spongy lesion that occurs in its typical form, perhaps exclusively, in subacute bacterial endocarditis. Through the presence of certain histological stigmata of rheumatic fever it is possible to recognize those cases of bacterial endocarditis that are superimposed on previous rheumatic damage. In our material 75 per cent of the hearts had been the seat of a previous rheumatic process. In a discussion of the mechanisms involved in the implantation of bacterial endocarditis, reasons are given that indicate activity of a rheumatic infection is not a necessary precursor to the development of bacterial endocarditis. Aschoff bodies were encountered in approximately 30 per cent of the superimposed cases of acute and subacute bacterial endocarditis, and evidence is presented that supports the view that some of these cases were thrown into activity by the superimposed bacterial infection rather than that

the activity of the rheumatic process predisposed to the bacterial endocarditis. Certain mechanisms are suggested as the means by which the endocardial structures are predisposed to a bacterial implantation. These mechanisms include the formation of eosinophilic necrosis of the valve closure line, and thrombotic, proliferative and necrotic changes at these sites. Some of these alterations are brought about by the hemodynamics present in congenital and acquired defects. Others are probably due to inflammatory, toxic or degenerative processes. The endocardial alterations, together with intracardiac tension, seem to predispose the endocardial structures of the heart to bacterial implantation by providing suitable means for anchoring transient bacterial invaders. Some of these mechanisms are present in non-rheumatic valves, but less frequently than in rheumatic valves. It does not appear that the vascularization occurring in rheumatic valves plays an appreciable rôle in the implantation of bacterial endocarditis.

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## DESCRIPTION OF PLATES

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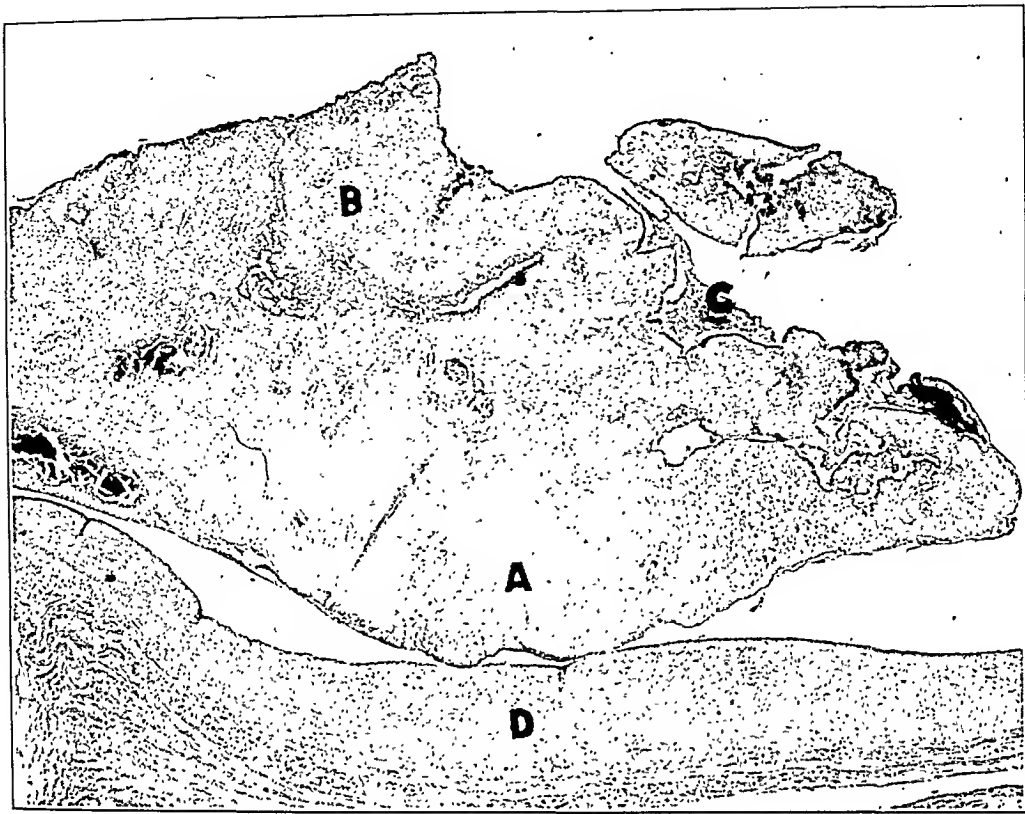
### PLATE 104

FIG. 1. Cross section of posterior aortic leaflet from a case of superimposed subacute bacterial endocarditis with negative blood cultures. Low power. Hematoxylin and eosin stain.

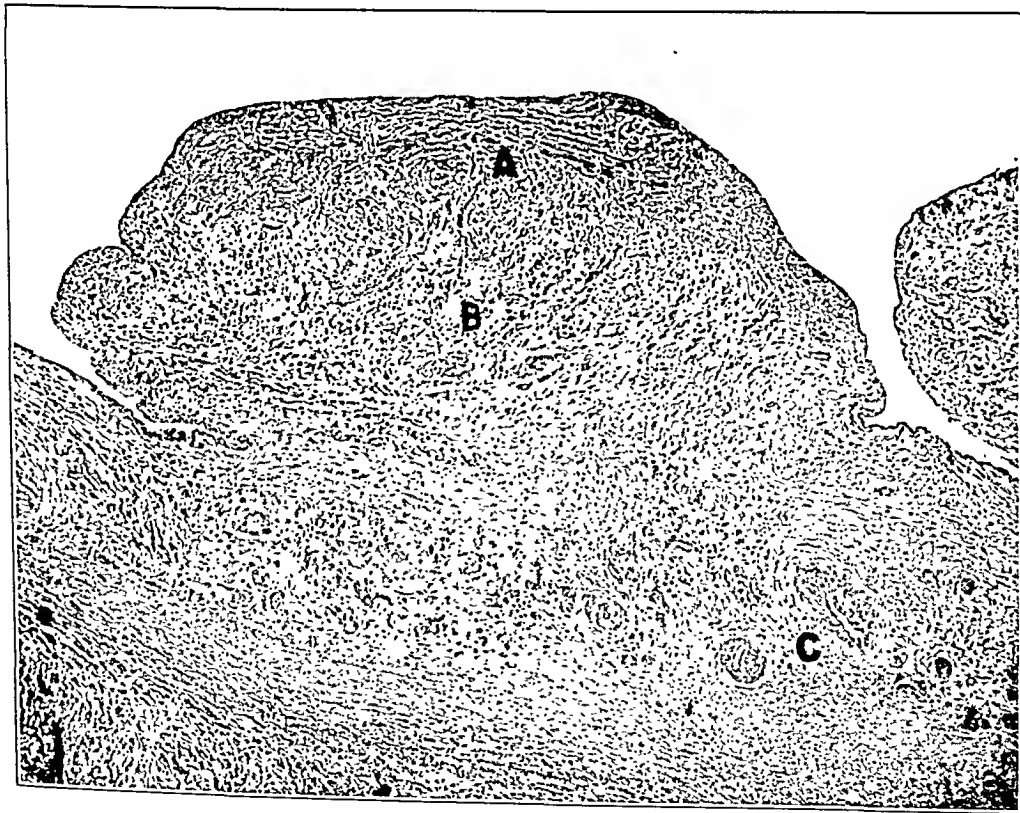
A = fibrosa layer of aortic leaflet; B = huge vegetations consisting largely of platelet structure and containing superficial and deep clumps of bacteria; C = fibrin; and D = aortic root.

FIG. 2. Organizing verrucous lesion on posterior mitral leaflet from a case of superimposed subacute *Str. viridans* endocarditis. Medium power. Hematoxylin and eosin stain.

A = verrucous material; B = extensive organization. (Note numerous capillaries and blood vessels.) C = muscular blood vessels in spongiosa layer of leaflet due to previous rheumatic process.



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PLATE 105

FIG. 3. Typical rheumatic verrucae on anterior mitral leaflet from an active case of rheumatic fever. Medium power. Hematoxylin and eosin stain.

A = close cropped verrucous material; B = active fibroblastic replacement at base. (Note very numerous large polygonal mononuclear cells.) C = intense capillarization of deeper layers of verrucae (part of the rheumatic process).

FIG. 4. Typical spongy lesion situated on the auricularis surface of the anterior mitral valve in a case of non-superimposed subacute bacterial endocarditis with negative blood cultures. Note large intercommunicating cavernous channels and lymphocytic reaction. Medium power. Hematoxylin and eosin stain.



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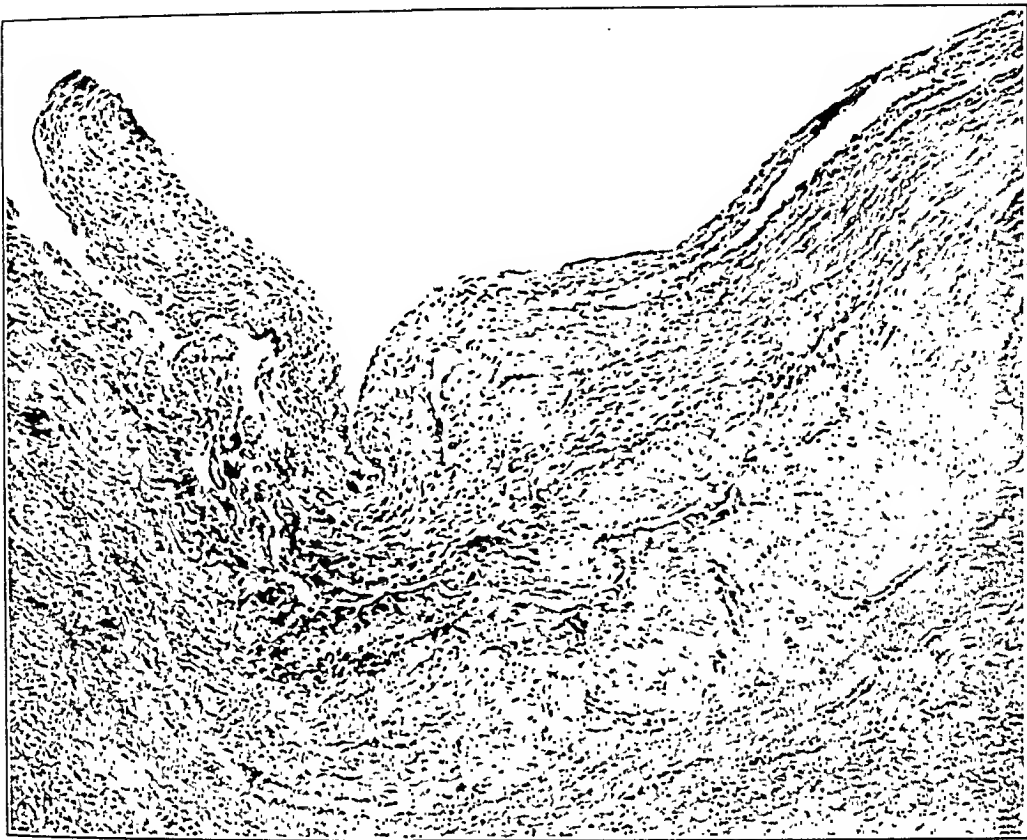
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PLATE 106

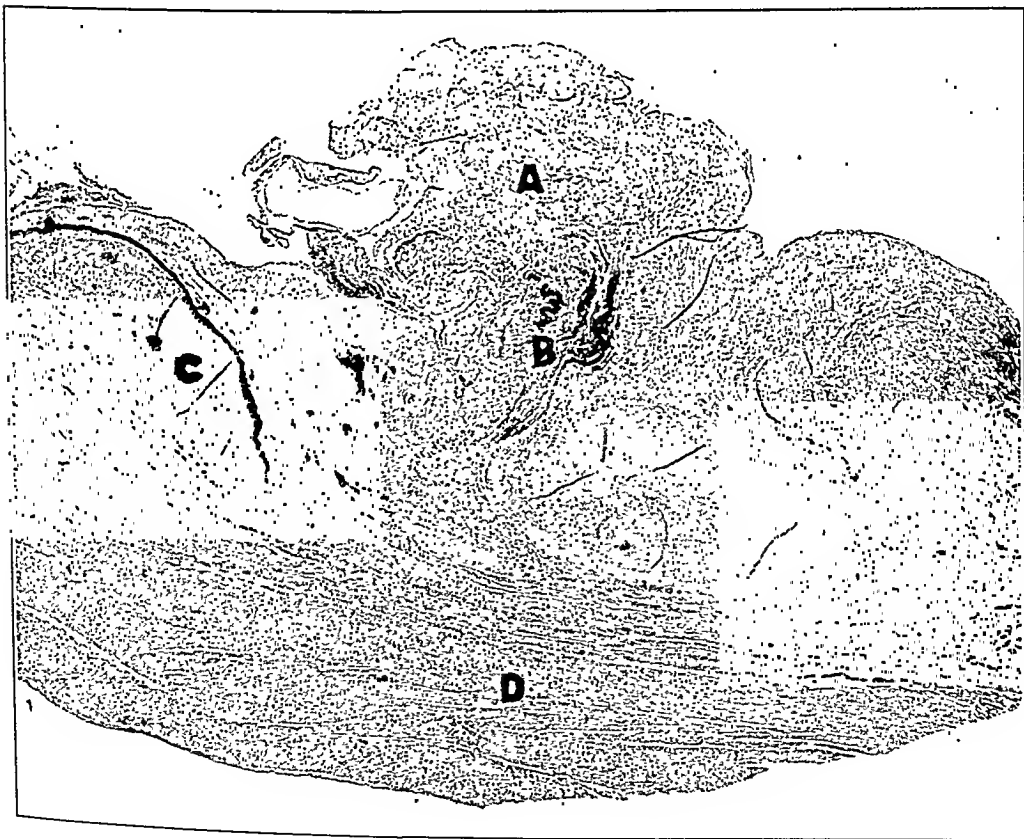
FIG. 5. Typical spongy lesion situated on the auricularis surface of the anterior mitral valve in a case of superimposed subacute bacterial endocarditis (bacteria-free stage). Note large intercommunicating cavernous channels and conspicuous fibrotic transformation. Medium power. Hematoxylin and eosin stain.

FIG. 6. Destructive bacterial process rupturing through base of aortic annulus in a case of superimposed subacute bacterial endocarditis with negative blood cultures. Low power. Weigert's elastic and van Gieson's connective tissue stain.

A = large fungating vegetation; B = ruptured elastic membranes from arterialis surface of annulus; C = lower portion of annulus; and D = myocardium from pulmonary conus.



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6



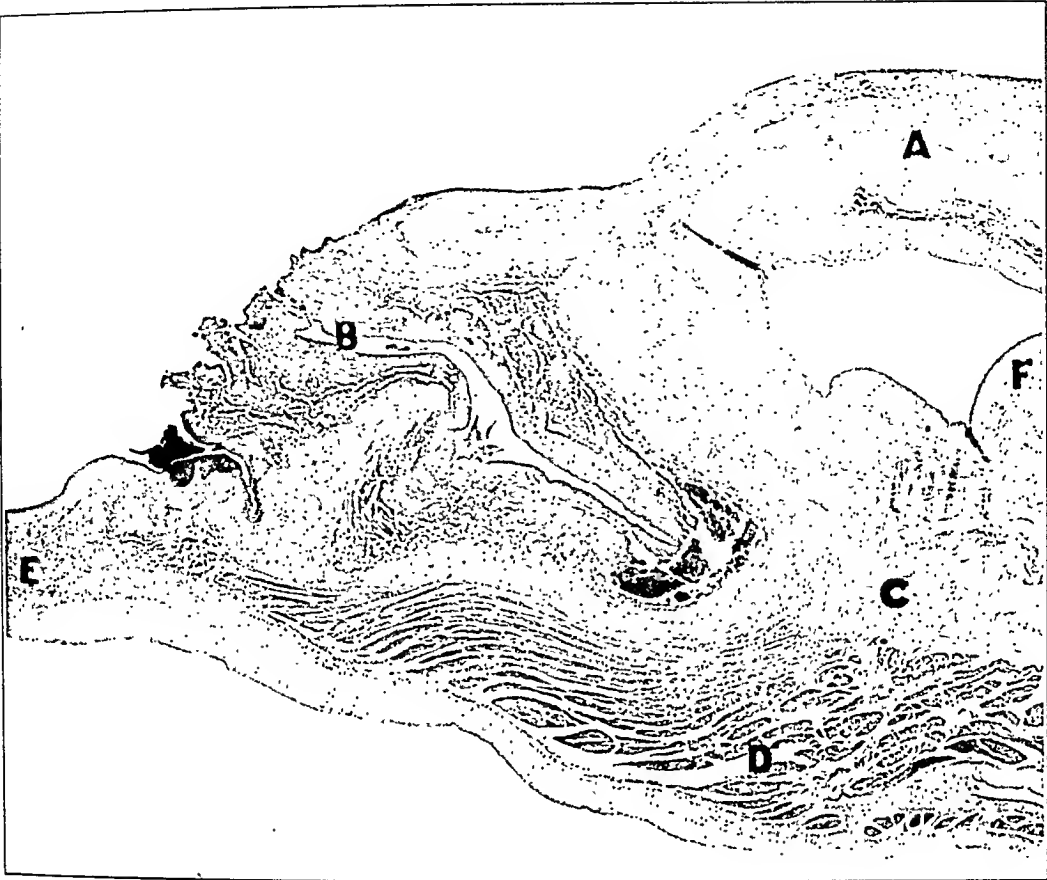
PLATE 107

FIG. 7. Rupture of bacterial process from subaortic angle toward retroaortic pericardial wedge in a case of superimposed subacute bacterial endocarditis with negative blood cultures. Low power. Hematoxylin and eosin stain.

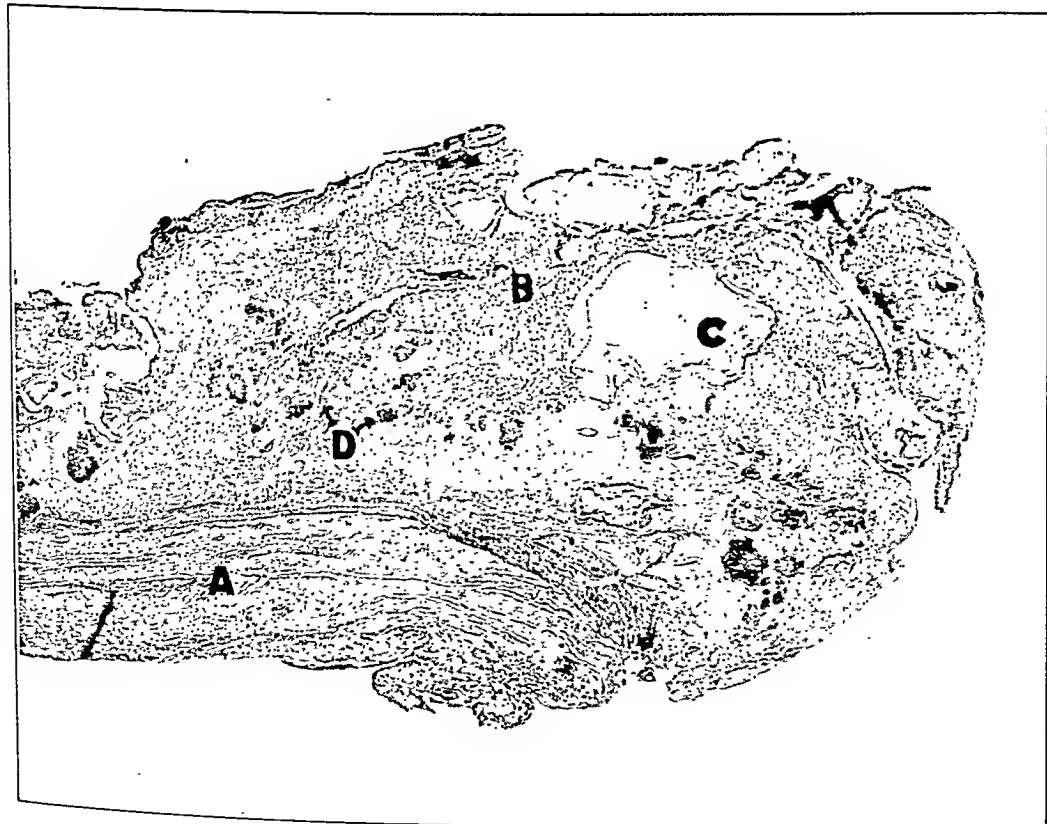
A = right aortic cusp; B = vegetation; C = retroaortic pericardial wedge; D = left auricle; E = mitral valve; and F = aortic root.

FIG. 8. Tip of right aortic cusp from a case of superimposed subacute bacterial endocarditis (bacteria-free stage). Low power. Weigert's elastic and van Gieson's connective tissue stain.

A = fibrosa layer of aortic cusp; B = fine granular material probably representing non-viable bacteria; C = calcific plaque; and D = bacterial clumps embedded in collagenous tissue.



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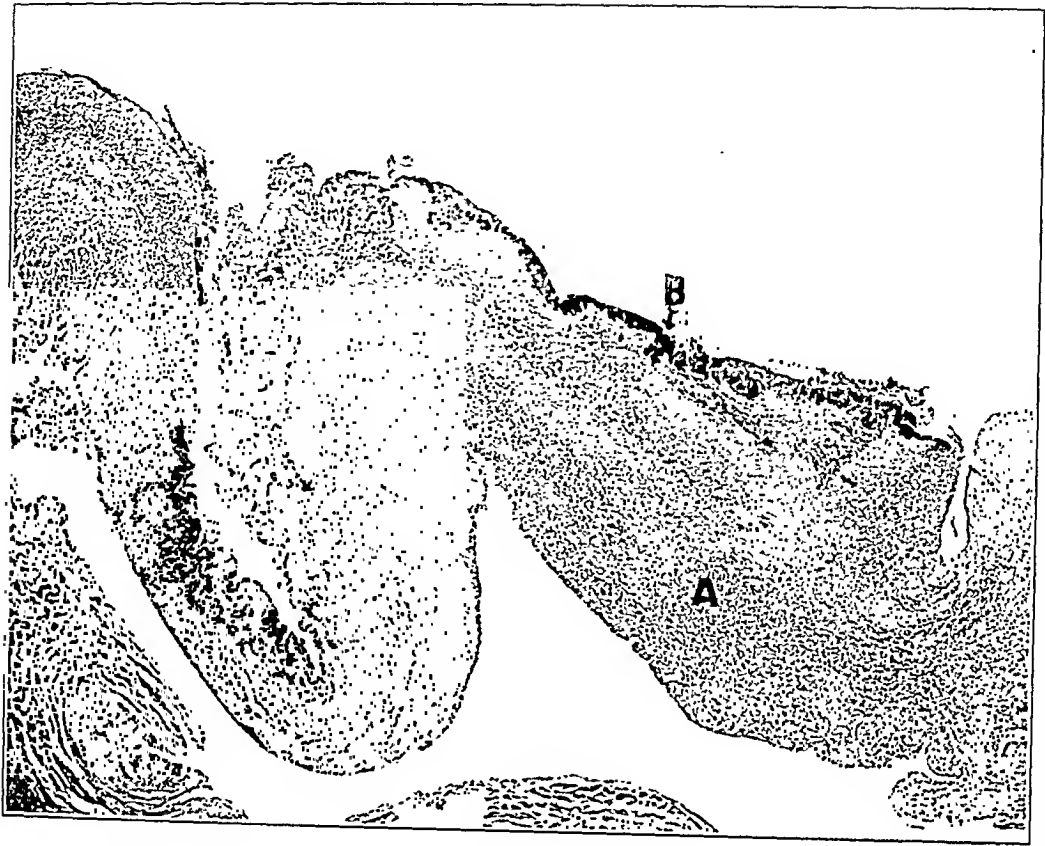
PLATE 108

FIG. 9. Tricuspid leaflet from a case of non-superimposed acute bacterial endocarditis (*Staph. aureus*). Low power. Hematoxylin and eosin stain.

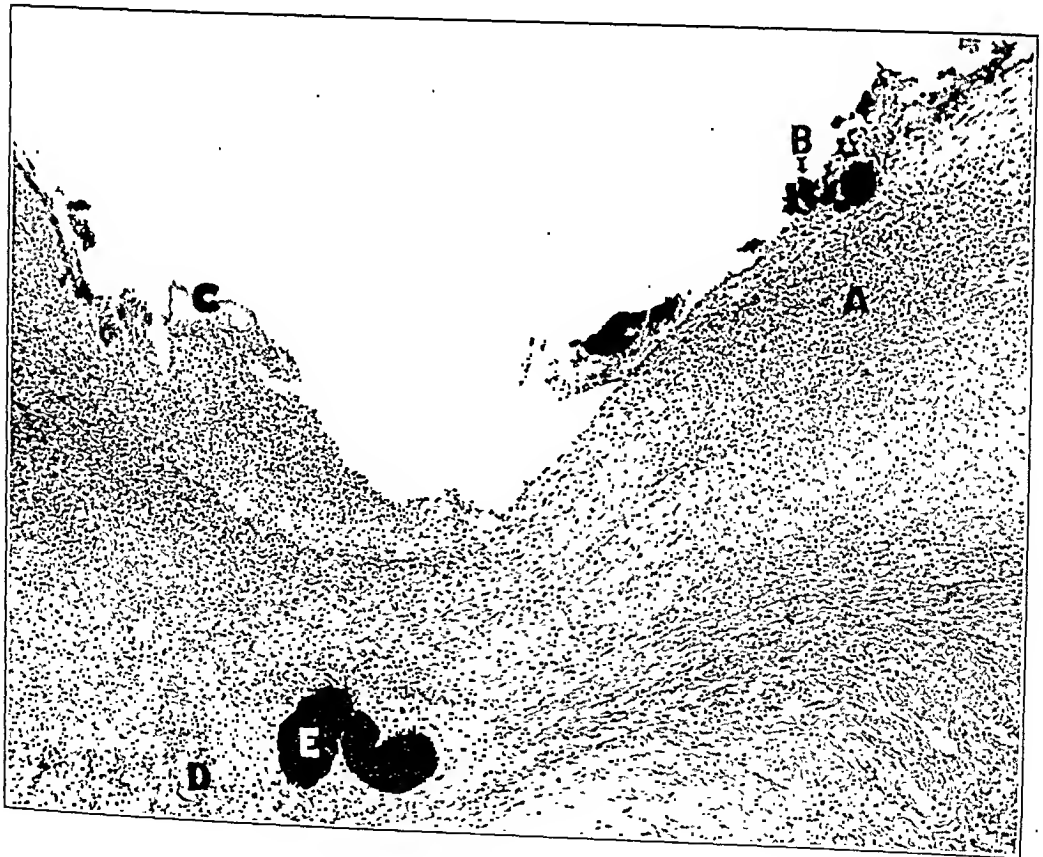
A = intensely necrotic valve leaflet; B = flat superficial bacterial lesion on auricular surface of leaflet.

FIG. 10. Angle of posterior mitral leaflet and left auricle from a case of superimposed acute bacterial endocarditis (*Staph. aureus*). Medium power. Hematoxylin and eosin stain.

A = intensely inflamed and necrobiotic valve leaflet; B = superficial bacterial masses; C = non-bacterial vegetation; D = intensely inflamed ring; and E = large blood vessel (injected) due to previous rheumatic process.



9



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## THE EFFECTS OF COAL SMOKE OF KNOWN COMPOSITION ON THE LUNGS OF ANIMALS \*

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The respiratory disease problem, as related to smoke and dust, depends on the degree and kinds of air pollution and varies with the proximity to, and the nature of, the industrial sources in the district. In any large city the problem divides itself into occupational and non-occupational forms of lung damage.

Occupational lung damage depends on exposure to a variety of dusts, organic and inorganic, and varies in degree with the different kinds and concentrations of dust and length of time of exposure. While many dusts may be irritating or produce sensitization, it is now generally conceded that the only inorganic industrial dusts capable of producing disabling fibrotic changes in the lungs are those containing free quartz, mixtures of free quartz with other substances such as coal dust, iron dust, aluminum, and so on, and asbestos. In addition, it is necessary that these dusts be in concentrations greater than 50 million particles per cubic foot containing 5 per cent of free quartz under 10  $\mu$  in diameter, or 10 million particles with 35 per cent quartz.<sup>1</sup> Exposures to lower concentrations require longer periods of time than those containing less quartz. If emphasis is placed on the qualifying term "disabling fibrotic lung changes," we concur in this opinion but, based on pathological changes in anthracotic lungs and on experimental results, we take exception to the frequently published statement that carbon in itself is incapable of producing appreciable degrees of fibrosis of the lungs.

In general, non-occupational lung changes are those found in the inhabitants without regard to occupation. They vary with

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the concentrations of air contamination of the district and the length of residence.

Coal smoke is the most common type of general air pollution, and its chief particulate constituents are carbon and non-crystalline ash. The silica content of coal smoke, according to our analysis, is far below the levels generally considered injurious, and yet it is a well recognized fact that practically all adult inhabitants of smoky atmospheres show obvious gross and microscopic evidence of particulate deposits, and varying degrees of perilymphatic lung fibrosis.

Reports based on the studies of the relation of lung fibroses to other diseases among industrial groups (see Collis,<sup>2</sup> Sayers,<sup>3</sup> and Lanza<sup>4</sup>), in comparison with the reports made in non-occupational groups and in soft coal miners (see Klotz,<sup>5</sup> Haythorn,<sup>6</sup> Schnurer *et al.*,<sup>7</sup> and Hayhurst<sup>8</sup>), indicate one chief practical difference between the two groups: pulmonary fibroses due to, or associated with, silica dusts in occupational groups predispose to tuberculosis, while fibroses due to pigmentation in non-occupational groups do not. The opposite finding appears to be true with reference to pneumonia. In Pittsburgh, for example, where the inhabitants with non-occupational lung changes far outnumber the industrial employees with more severe forms of lung fibrosis, the pneumonia death rate is usually in the neighborhood of 40 per cent higher than for the Commonwealth of Pennsylvania as a whole. The recent final report for the year of 1935 gave the Allegheny County pneumonia death rate as 121.9 per 100,000 and the State rate as 83.6. This condition has persisted year after year. Although Schnurer has found pneumonia more common in the higher grades of anthracosis than in the less severe, no entirely satisfactory explanation has been found for the locally high occurrence of pneumonia.

It has long been our desire to produce lung changes experimentally which simulate those of the non-occupational types and to study their relation to experimentally produced pneumonias.

Up to the present, the experimental production of the non-occupational types of lung damage, usually called anthracosis but perhaps more correctly termed bituminosis, has generally been unsuccessful save in experiments where free quartz has been

mixed with the carbon. The chief difficulty appears to have been the failure to get sufficient quantities of particulate carbon into the air sacs. Haythorn<sup>6</sup> in 1913 reported the production of fibrous proliferation in the lungs of guinea pigs insufflated with finely powdered, thoroughly dry lampblack. The experiments were carried to the point where several rows of carbon-filled phagocytes had collected in the perivascular lymphatics and had become compressed into spindle shapes by the formation of collagen strands between them. A similar picture was obtained by injecting carbon into the subcutaneous tissues of rabbits' ears. Haynes,<sup>9</sup> cited by Belt,<sup>10</sup> showed by animal experimentation that several dusts usually classed as non-dangerous, including charcoal and colloidal coal, produced proliferative lesions in guinea pigs' lungs indistinguishable from the early lesions of silicosis. Kettle<sup>11</sup> in 1934, in discussing dangerous dusts says, "I have myself seen pleural drift, plaque formation and a moderate degree of fibrosis, that is, the accepted signs of incipient pneumoconiosis with such an inert substance as carbon (India ink)." Opposed to these views are the reports of several French workers. Feil<sup>12</sup> cites Claissi, Josué, Jötten and Arnoldi, all of whom attempted but failed to produce fibrosis in animals exposed to smoke dust. Still others are quoted who claim that lungs blackened by pure coal are never fibrosed. Cummins and Sladden<sup>13</sup> in a comprehensive review of Continental work cite, and are in complete accord with, the results of Stern,<sup>14</sup> whose animal experiments showed that "coal dust alone will cause neither fibrosis nor cavitation."

In America, Willis,<sup>15</sup> reporting on the exposure of guinea pigs to finely sifted coal dust, finds that "fibrosis develops neither within the lungs nor in the tracheo-bronchial lymph nodes after one year of exposure," and concludes that "coal has but slight irritative properties." Lemon and Higgins,<sup>16</sup> speaking of pulmonary fibrosis, briefly dismiss the question with, ". . . and it is universally agreed that carbon dust, unless in large quantities, is of no pathological importance." Cummins and Sladden<sup>13</sup> take the position that anthracosis is a special variety of silicosis, depending on the simultaneous or subsequent accumulation of coal dust in the silica-damaged lung, and Sladden<sup>17</sup> in a more recent paper states that coal dust alone and apart from silica cannot be regarded as a cause of serious fibrosis of the lungs. In Gard-



ner's numerous articles on the effects of the exposure of lungs to various dusts we were unable to find a statement concerning carbon, save that he classified it among the inert dusts. In answer to a direct question he stated that he had obtained slight pneumonitis with an extremely mild proliferation of connective tissue after the inhalation and injection of carbon dust, but he considered this substance inert because the reaction was of such slight degree that it was only visible on microscopic examination. It should be noted here that the articles cited refer to "coal dust" and that our experiments were performed with "coal smoke" and, therefore, have to do only with the particles carried in suspension with the volatile portions of the smoke.

The present report is based on findings that developed in the course of a collaboration of studies made at the Mellon Institute of Industrial Research\* on the relative effects of the different kinds of fuels in common use on the lungs. The several kinds of fuel under study were burned in weighed amounts (1000 gm. per hour) continuously in egg stoves for a period of 80 days. Smoke from the flues of each stove was handled separately. One-fourth of the smoke was diverted and mixed with oxygen so that the oxygen content was constantly kept above 19 per cent. The smoke mixtures passed through gas-tight chambers of 60 cubic feet capacity. Four rabbits and 8 rats were placed in each compartment. The controls were placed in a chamber of like construction which was supplied constantly with clean filtered air. For the purposes of this paper, the animals in the bituminous and control chambers are all that need be considered.

The air-gas contents in the chambers were changed completely every 15 minutes. Regular analyses of the gas contents were made. The amounts of sulphur dioxide and carbon dioxide were kept below any percentage reported as toxic, and carbon monoxide

\* The experiments were made during the summer and autumn of 1935 as a part of the Anthracite Institute Fellowship at the then uncompleted new Mellon Institute of Industrial Research at Pittsburgh. The engineering features were conducted by members of the research staff of the Institute. The set-up of stoves, the means of adding air and oxygen and of regulating the amounts of flue smoke, the collection of chemical samples and the chemical analyses, were under the direction of H. B. Meller, Head of the Air Hygiene Investigation of the Mellon Institute, and M. A. Yavorsky. A report of the medical phases has been completed by Schnurer and is ready for publication. The animal chambers were borrowed from the United States Bureau of Mines.

did not exceed more than a trace at any time. The animals were kept in the chambers 23 hours out of every 24 (Table I).

TABLE I  
*Gas-Air Mixtures in Chambers*

	O <sub>2</sub>	CO	CO <sub>2</sub>	H <sub>2</sub> S	SO <sub>2</sub>
	%		%		%
Maximum	20.8	Trace	10.0	Trace	13.63
Minimum	19.2	"	0.2	"	3.33
Average	20.0	"	0.6	"	7.51

Dust counts were made regularly by Yavorsky, whose results are shown in Table II. Records of the temperature and humidity in each chamber were kept throughout the exposures.

TABLE II  
*Dust Counts \**  
*Particles per Cubic Centimeter †*

	Maximum	Minimum	Average
Control	221	38	125
Bituminous coal	5640	3502	4410

\* Owens Jet Counter at 970 X.

† 4410 particles per ccm. = 125 million per cubic foot.

Dr. R. R. Ruppert of the Mellon Institute determined the silica content. The following paragraph is a summary of his report.

"The free silica content of the bituminous coal soot collected in the course of the work was determined, using microincineration to determine the ash in the soot by particle count and the petrographic method of immersion in liquids to find the free silica content of the ash. Using a high magnification, similar to that used in making the dust counts in the course of the tests, the free silica particle content of the soot was found to be 0.8 per cent. That figure multiplied by the dust content of the atmosphere taken in the course of the work gives 0.4 million particles of free silica per cubic foot of air, to which the animals were exposed. That concentration is well below the danger limits, and accord-

ing to any established authority the silica is not present in an amount sufficient to cause fibrosis."

Blood counts were made at weekly intervals. Whenever an animal died an autopsy was performed promptly and tissues preserved for microscopic examination.

### MICROINCINERATION

Lung sections from animals in each group of experiments were incinerated at 400° C., treated with dilute hydrochloric acid and examined by polarized light under a metallurgical microscope. No highly refractive crystalline particles similar to those found in the incinerated sections of silicotic lungs could be demonstrated. The former positions of the sections were outlined by a fine, gray amorphous ash.

### PATHOLOGICAL EFFECTS

In the bituminous chamber 2 rabbits died of bronchopneumonia before the 80 days of exposure were completed. Immediately after the exposure 1 of the 2 remaining rabbits and 6 of the rats were killed and autopsied. The rabbit had a purulent bronchitis and areas of bronchopneumonia. The pleura was diffusely blackened as well as spotted with black pigment and, on section, black blotches appeared on the cut surface throughout the lung tissue. The peribronchial lymph nodes were enlarged and blackened. All of the rats had bronchitis, although no consolidation was found in any of them. The lungs and peribronchial nodes were black or reddish black in color.

Microscopically the findings in the rabbit and rats were similar and separate descriptions would be unnecessary repetition. In all of them the bronchial and bronchiolar goblet cells were swollen and active. A polymorphonuclear and mononuclear cell exudate was often present, and free black pigment and pigment-bearing phagocytes were abundant. The infundibular and alveolar contents varied greatly; some were packed with free pigment and pigmented phagocytes, some contained relatively small numbers of phagocytes, and some were empty. In the areas where pigment was greatest in amount the alveolar walls had collapsed about it and compressed it into masses. The walls of the collapsed air

sacs were congested, thickened, and filled with mononuclear cells and leukocytes. Agglomerations of pigment phagocytes were present in the air spaces and were sometimes fused into multinuclear giant cells. Single pigmented cells were sometimes present in the trabeculae, and collections of them were gathered about the peribronchial, perivascular and subpleural lymphatics. The condition at the end of 80 days was that of a focal interstitial pneumonitis with little fibrous proliferation. In the lymph nodes the pigment was practically all intracellular and the cells were for the most part loosely distributed throughout the lymphoid tissue.

One of the 4 control rabbits died of bronchopneumonia during exposure. The other control animals, killed at the end of the experiment, were either pigment-free or presented a few, widely separated pigment phagocytes in the air sacs.

A rat from each group was killed 103 days after the experiments were discontinued. The control in this instance was essentially normal as far as pigment was concerned. The lungs of the rat from the bituminous chamber were deeply pigmented. Grossly the pleura was studded with black spots having bright, glistening emphysematous centers.

Microscopically these sections resembled those of the animals killed after exposure save that almost no pigment remained free. There were fewer pigment phagocytes in the alveoli and a great many more in the perivascular lymph spaces than in the previous specimens. The blackened patches, seen when the section was held to the light, were made up of groups of alveoli filled with masses of pigmented cells, and the intervening walls showed definite infiltration with phagocytes and clear mononuclear cells (pneumonitis). Fibroblasts in these areas showed a definite increase in number and filamentous strands of collagen, stained blue by Mallory's aniline blue connective tissue stain. In the lymph nodes the pigmented cells were collected in clumps and in fused masses.

The most important results were those in the final rabbit from the bituminous chamber which was killed 429 days after the exposure. Grossly the lungs and pleural surfaces were spotted with discrete black blotches distributed in a pattern similar to that seen in a soft coal miner's lung. The peribronchial lymph nodes were very black and were somewhat enlarged.

Microscopically the findings were milder than those found in

bituminosis and more nearly resembled the condition found in the non-occupational forms of anthracosis. The blackened patches were much like those found in the animals described above except that the foci were more clean-cut, more discrete and more widely separated, while the fibrous proliferation was distinct and unmistakable. The phagocytic collections about the vessels were several rows in thickness and, while they were not compressed into nodules, they were often separated from each other by collagenous strands of varying widths. Local areas of emphysema were often found in the immediately surrounding lung tissue, and there were many single large air sacs, so large that their diameters were equal to the thickness of the whole plaque.

In the lymph nodes the pigment cells had collected in patches and practically no separate unfused phagocytes remained. Under high power examination the patches were found to be unbelievably large fused giant cells, literally packed with black pigment. No collagenous bands similar to those found in the lungs could be demonstrated in or about the pigment masses in the lymph nodes.

## DISCUSSION

Rabbits and rats were exposed for 80 days in closed chambers to bituminous coal smoke containing an average of 125 million particles per cubic foot, of which only 0.4 million particles were free silica dioxide. Some of the animals were killed and examined immediately after the exposure, and the rest were returned to the animal house and subsequently examined at various periods for more than a year. The lungs of all of the animals were deeply pigmented, and the amounts and distribution of the black pigment were similar to that of moderate anthracosis. Microscopically the air sacs of the first animals examined were found to be filled with free pigment particles and phagocytes filled with carbon. The phagocytes tended to collect about the bronchioles and in the perivascular and subpleural lymphatics. In addition there were more or less isolated plaques or islands of alveoli completely filled with pigmented cells beneath the pleura and throughout the lung. The animals that were killed later showed practically all of the pigment to be within phagocytes. The collections of pigment in the lymph channels had increased. The plaques of massed carbon cells con-

tained active infiltrative pneumonitis, hyperplastic fibroblasts and a progressively increasing interstitial deposit of collagen. The appearance in the later animals varied from that seen in non-occupational anthracosis to that of the milder forms of bituminosis seen in soft coal miner's lung. Although the collagen was not laid down in whorls, considered by many to be typical of silicosis, the plaques themselves were nodular in structure.

The question then arose as to the nature of the stimulus responsible for the fibrosis. Gaseous elements and amorphous ash could be ruled out on the results of experiments carried out at the same time with coke and anthracite coal smokes in other groups of animals. These fuels produced the same gases and ash contents as soft coal and in amounts that were comparable, yet no pneumonitis or fibrosis was produced. Silica in extremely small amounts and carbon in relatively large quantities remained to be considered. As long as silica was present at all it was not entirely possible to refute the claims of the investigators who maintain the position that where "there is no silica there is no fibrosis." However, the quantity of free quartz was less than one-tenth of that amount stated in the definition of a safe dust. The period of inhalation was only 80 days, and no crystalline particles were found in the sections after incineration. The quantities of carbon were too great to be completely taken care of by phagocytes and, since it is not soluble, it remained in the lungs as a foreign body. That it was not entirely inert is shown by the cellular reaction about it in lungs that were otherwise free from pneumonitis. For these reasons we believe that carbon was responsible for both the pneumonitis and the fibrosis.

### SUMMARY

1. An experimental method is described by which finely divided carbon particles in an almost pure state may be successfully introduced into the alveoli of the lungs.

2. Immediately after exposure to the smoke inhalations the lung lesions were those of non-occupational anthracosis.

3. The animals kept under animal house conditions for several months to a year after exposure to smoke developed fibrous reactions about the carbon deposits with the formation of collagen

strands. The resulting lung changes were analogous to those of a milder grade of bituminosis as seen in soft coal miners.

4. The method provides a way of preparing lungs for the experimental study of the actual relation of carbon deposits to the common respiratory infections.

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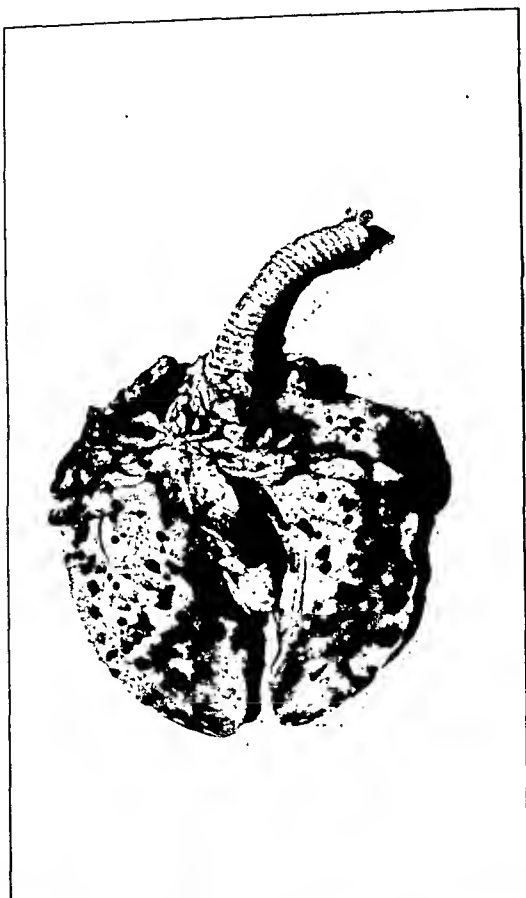


## DESCRIPTION OF PLATES

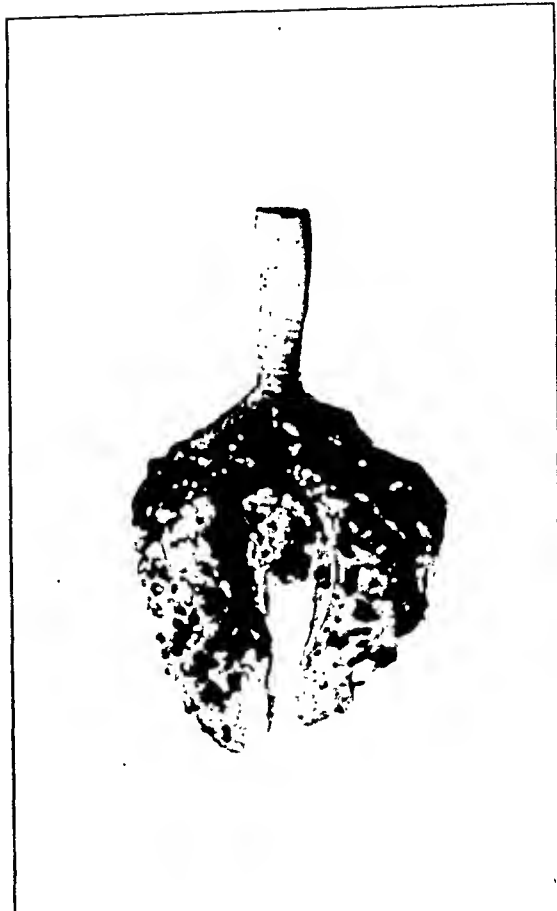
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### PLATE 109

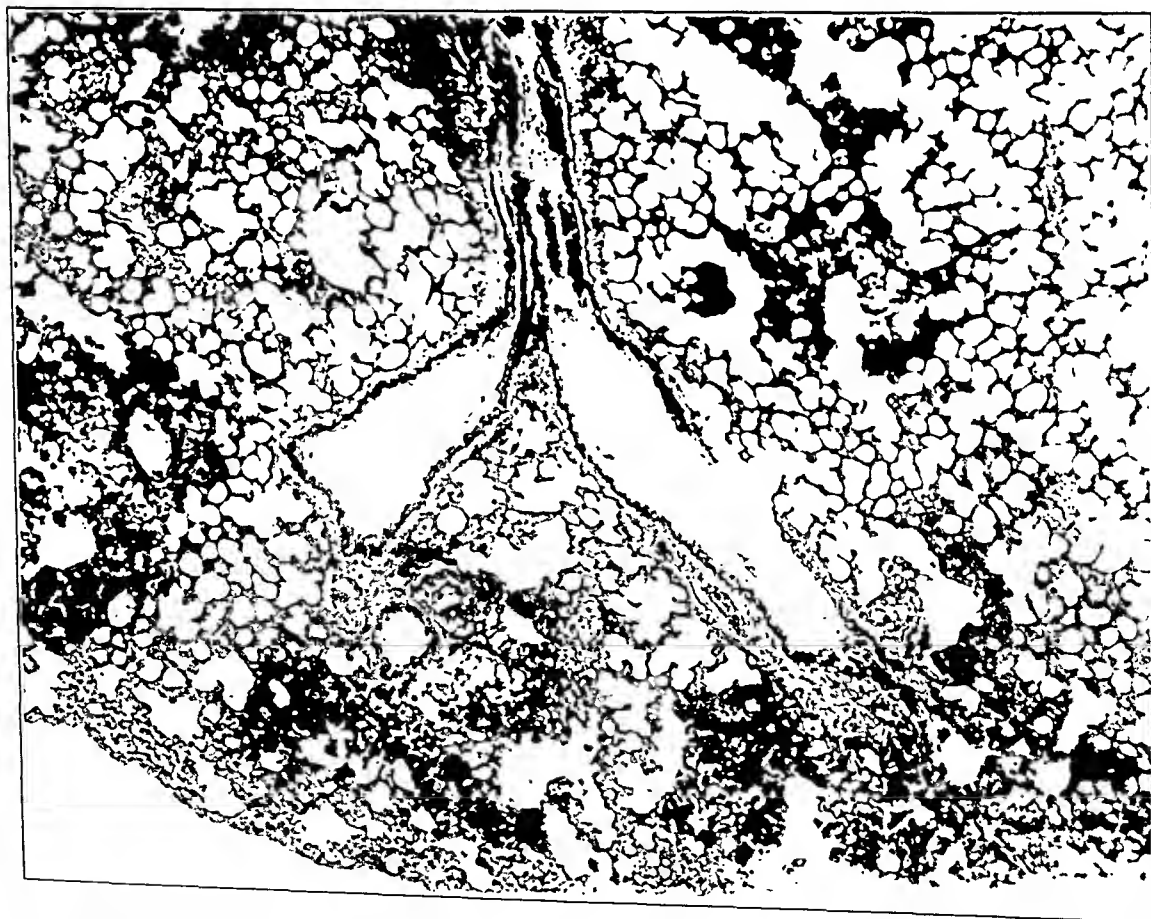
- FIG. 1. Gross appearance of the lungs of a rabbit exposed to bituminous coal smoke for 80 days and kept under animal house conditions for 1 year.
- FIG. 2. Gross appearance of the lungs of a rat exposed to bituminous coal smoke for 80 days and autopsied directly after the exposure.
- FIG. 3. Low-power section showing the wide distribution of carbon in the lungs of a rat exposed to inhalations of bituminous coal smoke for 80 days and autopsied at the end of the experiment.  $\times 20$ .



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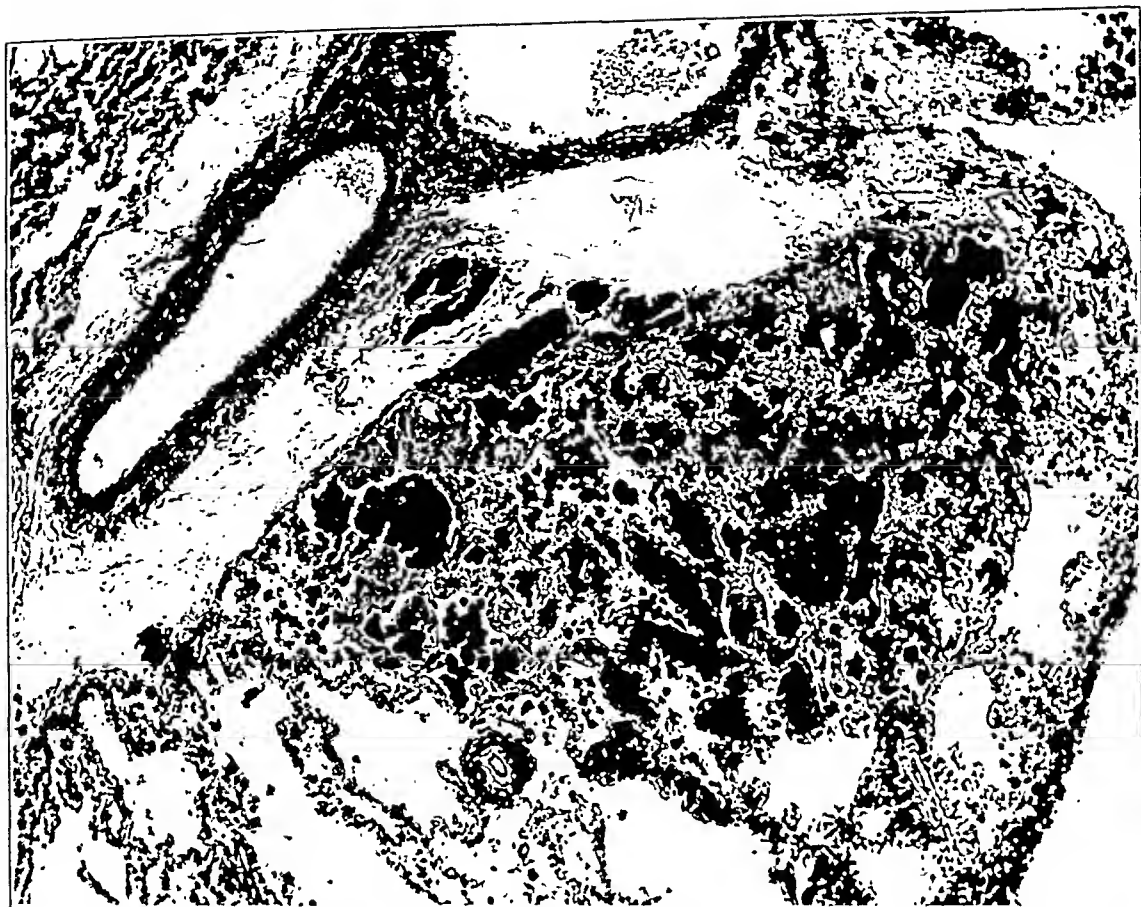


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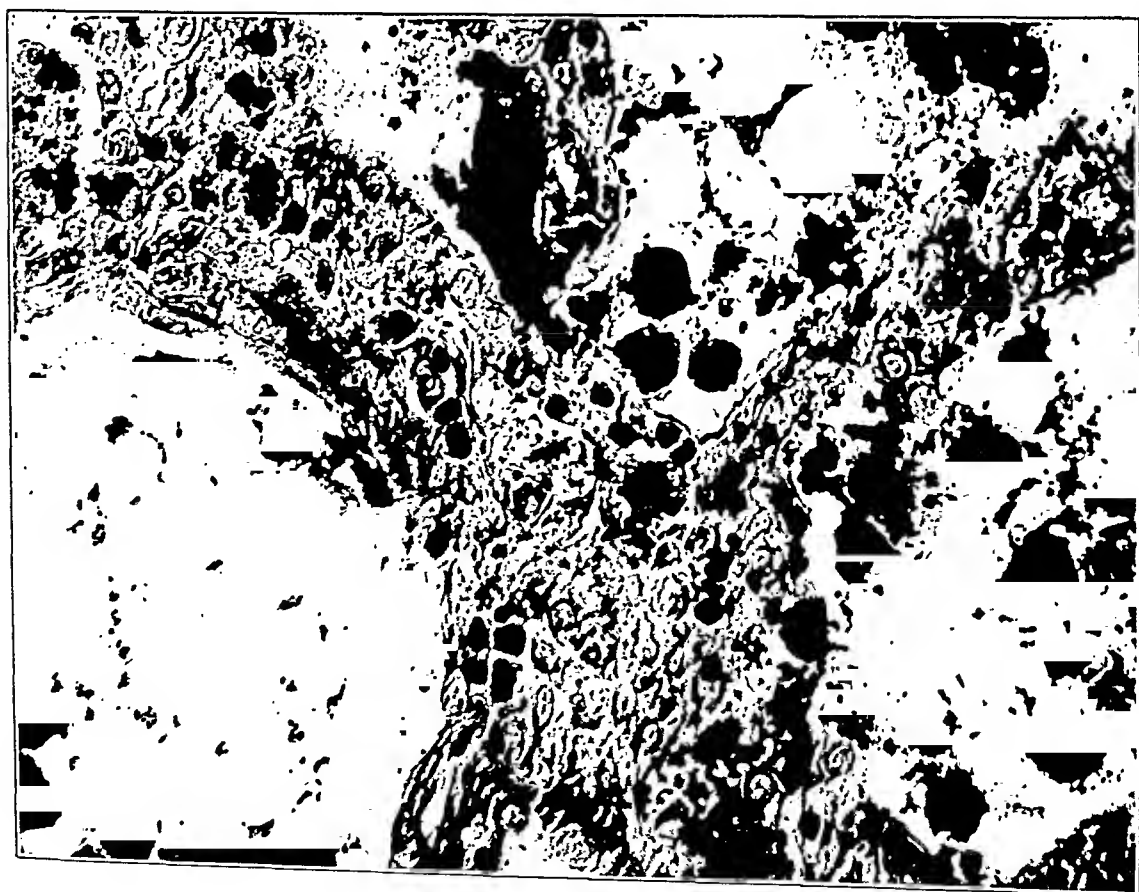
PLATE 110

FIG. 4. Low power section of a rabbit's lung exposed to bituminous coal smoke inhalations for 80 days and kept in the animal house for 1 year. Note the thickened alveolar walls in the pigmented area and the rows of carbon-bearing cells in the perivascular lymph spaces.  $\times 150$ .

FIG. 5. High power section taken from the nodules shown in Fig. 4 to show interstitial pneumonitis, hyperplasia of the fibroblasts and the deposit of collagen material.  $\times 350$ .



4

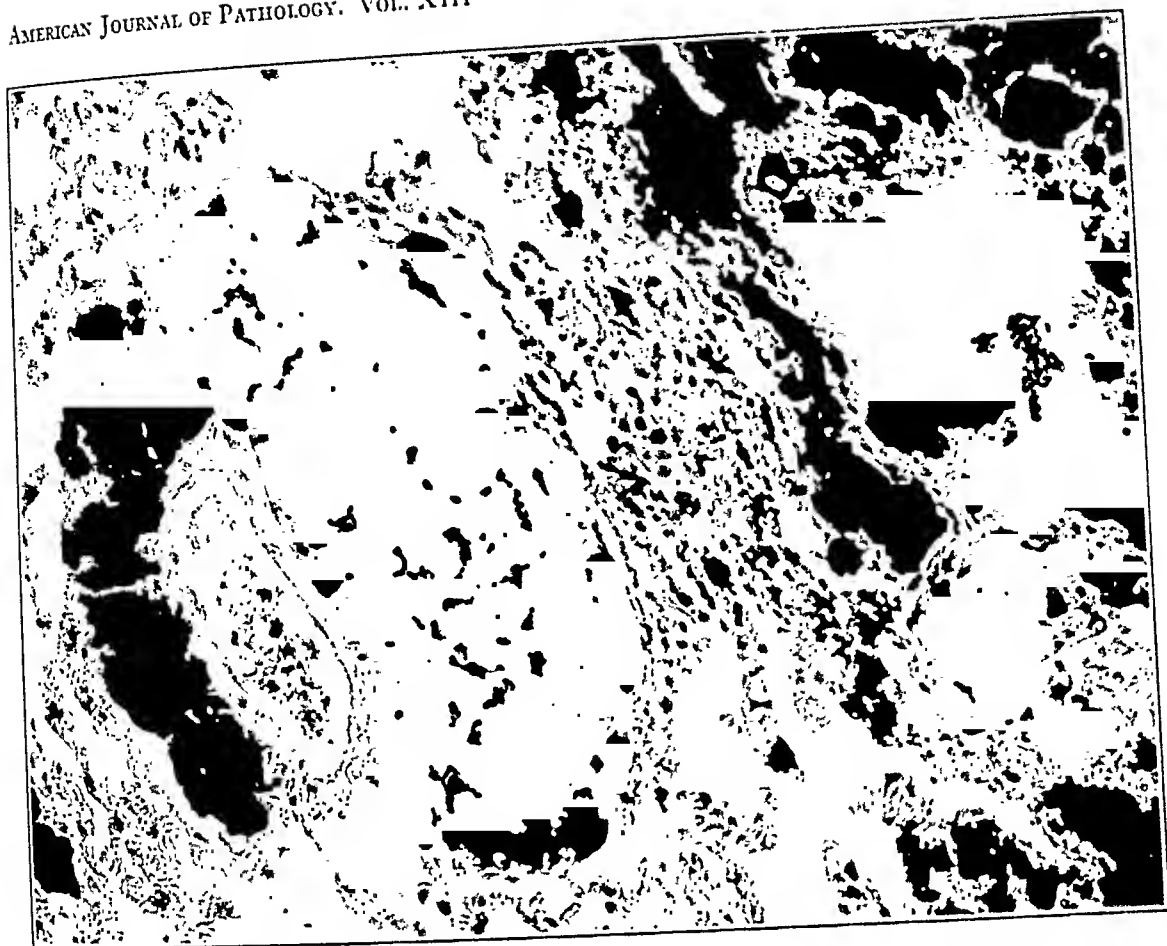


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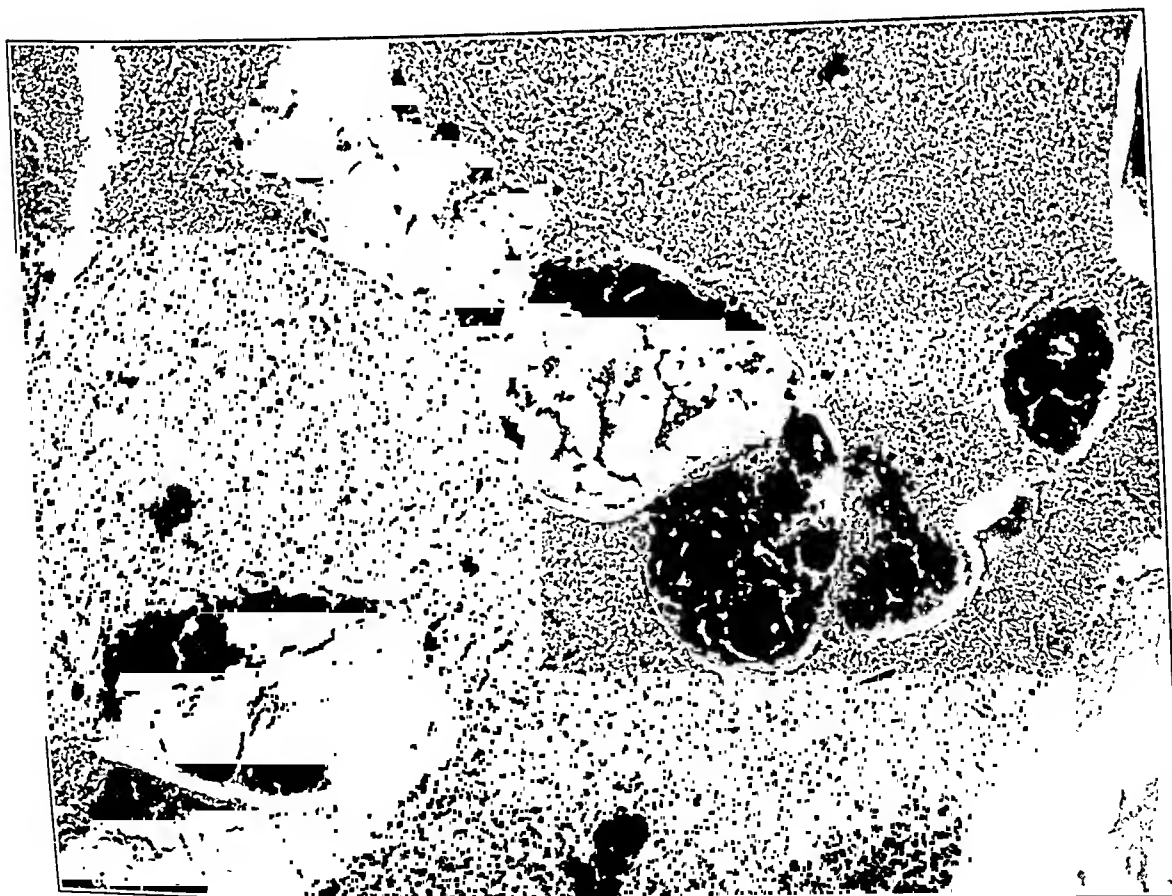
PLATE III

FIG. 6. High power section from the same animal showing the compact pigment deposits in the alveoli and the rows of carbon cells in the perivascular lymphatics.  $\times 200$ .

FIG. 7. Low power section of peribronchial lymph node showing the accumulation of pigment phagocytes in masses. Many are fused to form large multinucleated giant cells.  $\times 150$ .



6



7



# THE POSTMORTEM ELASTICITY OF THE ADULT HUMAN AORTA. ITS RELATION TO AGE AND TO THE DISTRIBUTION OF INTIMAL ATHEROMAS \*

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The association between the elastic properties of arteries and the development of the intimal lesions of arteriosclerosis has never ceased to intrigue interest. The existence of such a relation appears to have been first stressed by Polotebnow<sup>1</sup> in 1868 who observed that a sclerotic femoral artery could be stretched only one-fourth as much as a normal one. The same facts are apparent from the protocols of Wertheim,<sup>2</sup> 1847, and Wundt,<sup>3</sup> 1858, although these authors were more concerned with the physiological nature of arterial elasticity rather than the relation of the latter to disease. That the aorta undergoes a progressive enlargement throughout adult life in both length and circumference is well known. Kaufmann,<sup>4</sup> who made extensive measurements on a large number of aortas, found that the size of the aorta depended chiefly on age and body build. This enlargement with age, it is generally conceded, is associated with a loss of resilience and ability to stretch with traction. Such changes are not necessarily associated with the intimal lesions of true arteriosclerosis but are usually designated as old age phenomena, the result of degeneration of elastin, with alterations in its colloidal properties, and consequently reduced elasticity.

Nevertheless many attempts have been made to demonstrate a relation to the development of intimal atheromas. The various viewpoints are reviewed in surveys by Wells<sup>5</sup> and Aschoff.<sup>6</sup> Perhaps a majority of authors would deny the existence of any other than accidental chronological association. However, the theories of Thoma and his school, that loss of elasticity and weakening of the vessel wall are the primary factors that precipitate all subsequent intimal lesions, have never wanted for ardent supporters

\* Received for publication July 2, 1937.



even in recent years. Wells,<sup>5</sup> for example, in a discussion of the chemical changes in arteriosclerosis comments: "It appears that a consideration of the chemical and particularly the physicochemical properties of arteries lends support to the view that arteriosclerosis depends primarily on changes in the elastic tissue that reduce its resilience and lead to arterial dilatation. The subsequent changes seem to be secondary to this yielding of the media." He further quotes Klotz and Beitzke as supporting the views of Thoma in their chief details.

Efforts to measure a possible connection from the physical properties exhibited by arteries have been conducted in two major ways. The degree of elasticity of excised vessels has been measured directly and coefficients of elasticity have been computed from the velocity of pulse wave transmission in the living subject. The early literature on the first of these two methods has been thoroughly and critically reviewed in the extensive monograph of Reuterwall.<sup>7</sup> Only the salient features will be mentioned here.

One group of authors, Moens, Roy, Herringham and Wills, Israel, and Suter (cited by Reuterwall<sup>7</sup>), and more recently Yater and Birkeland,<sup>8</sup> employed narrow strips of aorta of known length and width and measured elongation when these were subjected to variable degrees of traction. Roy<sup>9</sup> graphically recorded elasticity curves and these showed a progressive decline with advancing age and a similarity in form to elasticity curves obtained by stretching the ligamentum nuchae. He also observed a difference in elasticity between longitudinal and transverse strips and noted that the media, when freed of a thickened unyielding intima, often exhibited greater elasticity than when stretched in the intact state. Herringham and Wills<sup>10</sup> found that the greatest loss of elasticity occurred in the fifth decade of life. They stressed the fact that only when intimal lesions were extensive was the reduction of elasticity greater than might be anticipated by age changes alone. Israel<sup>11</sup> found that a variety of disease processes might alter the degree of elasticity exhibited by excised strips of aorta. Suter<sup>12</sup> correlated the loss of elasticity with the enlargement in circumference that occurs with advancing age and also made the interesting observation that the circumference of the aorta when stretched to its maximum was almost constant at all periods of adult life, approximating 11 cm. in the ascending portion.

A variation of this method was to determine the changes in length that occurred when the aorta was freed from its natural attachments (Hiller,<sup>13</sup> Faber,<sup>14</sup> and Scheel and Tendeloo, cited by Reuterwall<sup>7</sup>). The findings of these authors were in general agreement. They found that up to adulthood there was an actual increase in the degree of retraction and thereafter a gradual decline until very late in life when the aorta sometimes was even longer after removal than *in situ*. Both Hiller and Faber thought that intimal plaques exerted little influence on this property of the aorta.

Using other methods some authors (Marey, Roy, Grumach, Zwardemaker, Thoma and his associates, Kaefer, Luck, Poper and Luz, Hurthl, MacWilliam, Strasburger and Pommack, cited by Reuterwall<sup>7</sup>) attempted to simulate conditions as they obtain during life. Unopened segments of arteries, usually medium sized ones, were distended with fluids under increasing pressures and the increase in volume or diameter of the vessel used as an index of elasticity. The results of these experiments, although adding more detail and perhaps more precise measurements, merely served to confirm the major findings of the previous methods. It was from such experiments that Thoma and Kaefer<sup>15</sup> elaborated their theories concerning the pathogenesis of the intimal changes.

The results of this experimentation are open to many serious criticisms if they are used to interpret the functional activities of the arterial system during life. For example, they neglect the role of living irritable cells, particularly smooth muscle, in the reactions of the vessel wall to pressure stimuli. They ignore the influence of the normal attachments and pressure relations of neighboring movable parts on vessel movements. Since the development of reliable methods of estimating the degree of arterial elasticity *in vivo* under natural conditions, notably by Bramwell,<sup>16</sup> and others, the use of excised blood vessels in this connection has been largely discontinued. Nevertheless the results of the *in vivo* experiments have corroborated many of the earlier findings on excised vessels, indicating that elasticity of arteries depends to a large degree on the inert elastic properties of the elastic fibers and that the results of *in vitro* experiments are not necessarily invalid. Moreover Bramwell obtained the same type of

elasticity curves when he applied his technique to excised arteries. The results of both his *in vivo* and *in vitro* experiments were in agreement with many of the early observations of Roy.

Other objections have been raised against the use of excised vessels for the determination of arterial elasticity (Reuterwall<sup>7</sup>). Many difficulties are encountered if absolute measures to conform to the physicist's concept of elasticity are attempted. Arterial elasticity does not seem to obey the same rules that govern the elastic properties of inorganic materials and even of other organic substances. For example, if different methods of measurement are used, quite different quantitative results may be obtained. Moreover, repeated tests on the same segment of artery wall may show quite variable results. The efforts to compare arterial elasticity to that of other substances have not been uniformly successful.

Another criticism is that there is no assurance that the elastic properties may not be so altered by events that transpire before and after death that it becomes impossible to compare the results obtained from different arteries. The actions of toxins, the effects of muscular contraction and postmortem changes have to be taken into account. Nevertheless, there are certain indications that the judicious use of *in vitro* determinations may be of some value. In the first place the results of many different observers have shown striking uniformity, indicating that the postmortem elasticity is no haphazard unpredictable property. Moreover, as already indicated, they agree in many respects with the *in vivo* findings. There is thus some justification for believing that if measurements of elasticity are conducted under identical circumstances, the results should be comparable to each other even if absolute values cannot be obtained and even if the results are not directly applicable to interpretations of the reactions of living vessels.

The application of *in vivo* measurements is limited in that they must be derived from relatively large segments of the vascular tree and cannot be directly associated with development of small intimal lesions. It is generally accepted that the intimal lesions show a definite predisposition to develop earlier and more frequently in certain parts of the adult aorta, namely in the posterior thoracic and lower abdominal areas (Aschoff<sup>17</sup>). Even when the

lesions become more widespread and the earlier pattern is lost, these regions are apt to bear the most advanced ones. If a loss of elasticity commonly precedes the development of these changes, it might be expected that these same areas would show greater losses than for example the more proximal regions of the aorta where atheromas are decidedly less apt to develop.

Moreover, the early work of Roy suggests that at least some of the loss of elasticity that accrues with age is not due to degenerative changes in the elastic fibers of the media but to the restraint exercised by a thickened unyielding intima. Obviously *in vivo* experiments cannot be used to determine the elastic properties of the various layers of the vessel wall, whereas this can readily be accomplished on excised vessels.

With these points in mind, a systematic study of the postmortem elasticity of the aorta was undertaken, first to compare the elasticity of various parts of the same vessel showing different degrees of intimal atheroma, and secondly to determine the role of the intima in producing an apparent loss of elasticity.

### MATERIALS AND METHODS

The aortas of 123 adults, which subsequently showed no microscopic evidence of disease other than arteriosclerosis, were removed in the usual fashion at autopsy. The individuals in this group had died of a wide variety of diseases and no attempt at selection was made other than to ensure a wide age distribution. The vessels were opened by a longitudinal incision along the posterior wall extending between the orifices of each pair of intercostal and lumbar arteries and washed freely in running water to remove blood clot. The adventitia was dissected away as completely as possible with scissors and forceps. The vessels were placed in 0.9 per cent saline and kept in the ice-box until at least 24 hours had elapsed after death in order to allow for a complete loss of smooth muscle irritability. All experiments were conducted within from 1 to 5 days postmortem. Little change in postmortem elasticity occurs during this period even if the aorta is allowed to remain in tap water at room temperature. At the end of such a period the tissue is obviously undergoing decomposition but will exhibit the same elastic properties as when first examined, indi-

cating that this property is not dependent on living cells and that the elastic fibers are quite resistant to postmortem autolysis. Similar observations were made by Roy and subsequently confirmed by many others.

Transverse strips 1 cm. wide were cut from the following areas: 1 from the ascending portion, 2 adjacent ones from the upper descending thoracic portion at the level of the 2nd and 3rd pairs of intercostal artery orifices, 2 adjacent ones from the lower thoracic portion directly proximal to the orifice of the coeliac axis, 1 from the upper abdominal region immediately distal to the orifices of the renal arteries and 1 from the lower abdominal region just above the bifurcation. All the strips represented the complete circumference except the ones taken from the ascending portion, which were sometimes incomplete. Four longitudinally directed strips, 1 cm. wide and about equal in length to the transverse thoracic ones, were cut from the midthoracic region of the descending aorta, 2 from the posterior half and 2 from the anterior half. In addition a single transverse strip 1 cm. wide was cut from the pulmonary artery just proximal to the obliterated ductus arteriosus.

The elastic property of each strip was measured in the following manner. One end of the strip was fastened by a small clamp with serrated jaws supported on a ring stand. A total of 300 gm. of weight was attached and suspended by means of a second clamp from the lower end of the strip for 1 minute. The stretched length was then measured to the nearest 0.05 cm.: 295 gm. were removed and the strip allowed to shorten for another minute and the length again measured. The degree of shortening that occurred when a strip stretched with a load of 300 gm. for 1 minute was subsequently allowed to retract against a load of 5 gm. for 1 minute was used as the measure of elasticity. This was expressed by dividing the actual shortening by the maximum stretched length. This figure multiplied by 100 gives the percentage of shortening.

It must be admitted that this is a highly arbitrary method of estimating elasticity and one that makes no attempt to imitate the natural conditions under which arteries are distended during life. For the purposes of this study a simple test which could be done rapidly under constant conditions on a large number of

segments was required. A 300 gm. weight was found to be great enough to overcome muscular rigor or contraction and to produce almost maximum stretching without tearing or traumatizing many of even the more delicate strips. Measurements on damaged strips were discarded. The final 5 gm. weight was used to keep the strip taut and prevent errors in measurement due to curling. The minute intervals were long enough to allow the tissue to come to equilibrium and short enough to prevent excessive drying. The tissue was allowed to reach room temperature before it was stretched. The degree of retraction rather than extension was used in order to eliminate unrecoverable yielding of the tissue not related to its elasticity. A stretched strip of aorta never quite returns to its original length. Only one test was performed on each strip. Where 2 adjacent strips were cut, 1 was tested in its intact state and the other after the intima had first been detached.

The method used for expressing elasticity requires some justification. Triepel,<sup>18</sup> studying the elasticity of a variety of animal tissues, elaborated a formula for the coefficient of elasticity in which this value varies inversely with the area of the cross section of the tissue tested. With lesser degrees of traction this quite likely holds true for aortic tissue as well, *i.e.* the resistance offered by aortic tissue to slight degrees of traction may increase with the thickness and width of the tissue. When, however, the tissue has been stretched almost to its maximum, as with a 300 gm. weight, subsequent retraction is simply due to the ability of straightened elastic fibers to recoil when released and is independent of the total number or amount of elastic fibers. This can be readily verified by subjecting 2 adjacent strips of aorta of equal length and thickness, but 1 strip 0.5 cm. wide and the other 2 cm. wide, to a 300 gm. traction. Both strips will stretch to an equal length and when the load is reduced to 5 gm. the degree of retraction is the same in both the wide and the narrow strips. In other words, the degree of retraction as measured under the conditions described is *not influenced* by the area of the cross section of the tissue. One cm. wide strips were used in order to limit rather narrowly each area to be tested and to facilitate the computation of intimal weight as described below. In further support of this contention are the observations of Yater and Birkeland<sup>8</sup> who employed strips of constant width and found that the elasticity was entirely inde-

pendent of the thickness. The use of Young's modulus \* to express elasticity is also not indicated for the same reason since the area of cross section is again a factor in this formula. Krafka<sup>19</sup> has calculated Young's modulus from the data of a number of other investigators and found that it showed little constancy and did not vary regularly with age.

The degree of intimal sclerosis was estimated by stripping the intima from the underlying media, obtaining the moist weight of the detached membrane to the nearest milligram. This weight divided by the length of the strip yielded the average weight of the intima per sq. cm. of surface for that particular portion. That the intima of the aorta is quite readily detachable from the underlying media was first noted by Roy. In most instances the separation is readily accomplished by peeling away the intima as an intact membrane which includes the entire bulk of any intimal plaques. The underlying media presents a smooth pale yellow surface. With care the separation of small strips can be accomplished with reasonable accuracy, experience showing when the underlying media is being torn since this tissue separates as thin elastic shreds and leaves a rough surface. Microscopic sections through such denuded vessel walls show a reasonably intact inner medial surface and sections of the detached intima regularly fail to reveal the presence of attached smooth muscle cells or elastic fibers. The only difficulties were encountered in cases of young individuals with delicate smooth intimas which were best separated by scraping with a knife blade, and in advanced intimal lesions where the plaques had encroached upon and become more firmly adherent to the media. But even in these instances it was felt that the weight of the intima could be obtained without undue error.

This gravimetric method of estimating the degree of intimal involvement over a small area offers obvious advantages to the usual subjective method of grading the lesions by mere inspection. The drawbacks to the method are that it measures diffuse intimal thickening not necessarily related to the atheromas and gives no exact idea of the type and nature of the lesions. As expected, the

\* Krafka uses the formula  $\frac{F \times L}{a \times dl}$  to express Young's modulus where F is the weight applied as traction, L the length of the test strip, a, the area of the cross section, and dl, the increase in length.

weight of the intima per sq. cm. showed a rough correlation to the macroscopic severity of intimal change. The delicate intima of young persons showing no plaque formation was about 20 mg. per sq. cm. The vast majority of normal intimas of all persons was in the range of 20 to 40 mg. per sq. cm., although occasionally, it was as high as 60 mg. per sq. cm. The presence of small lipoid streaks altered the weight only slightly. The presence of hyaline plaques produced a significant rise from 60 to 100 mg. per sq. cm., whereas the presence of grossly appreciable calcium deposits caused a sharp rise usually well above 100 mg. per sq. cm. It is thus seen that the more advanced lesions, namely, those showing hyalinization and calcification, were the ones that showed greatly augmented intimal weights.

## RESULTS

### *Relation of Age to Postmortem Elasticity of the Aorta*

In Table I the aortas are grouped by age in decades and the postmortem elasticity of the intact aorta is shown at the various levels tested. In the third decade the elasticity is fairly uniform at all these points and in both transverse and longitudinal planes. The posterior thoracic portion longitudinally and the lower abdominal segments have slightly lower values. This is a point of interest when it is recalled that these are precisely the areas in which the earliest atheromatous deposits are usually observed. With successive age groups there is a constant, fairly uniform decline in elasticity throughout life. The loss in longitudinal elasticity, particularly that of the posterior thoracic portion and of the abdominal portions transversely, proceeds more rapidly in the earlier decades of life and is always more severe than elsewhere. Later in life the other areas show greater reductions so that in the oldest age group there is less difference between the various levels tested.

The pulmonary artery in young persons exhibits less inert elasticity than the aorta. It too shows a progressive decline throughout life but one of much lesser degree so that in the oldest age group it has retained considerably more elasticity than any part of the aorta.



TABLE I

## Relation of Mean Postmortem Elasticity of Intact Aorta to Age

Area	Direction	20-29 yrs.		30-39 yrs.		40-49 yrs.		50-59 yrs.		60-69 yrs.		70-85 yrs.	
		No.	Mean % **	No.	Mean %	No.	Mean %	No.	Mean %	No.	Mean %	No.	Mean %
Ascending *** thoracic	Transverse	8*	37.4 $\pm$ 0.66	9	34.0 $\pm$ 0.64	10	31.7 $\pm$ 0.68	12	25.8 $\pm$ 0.97	10	23.0 $\pm$ 0.94	11	15.8 $\pm$ 0.93
Upper descending thoracic	Transverse	15	37.0 $\pm$ 0.46	13	33.7 $\pm$ 0.52	23	29.7 $\pm$ 0.42	21	27.0 $\pm$ 0.58	21	21.5 $\pm$ 0.48	18	16.2 $\pm$ 0.63
Lower descending thoracic	Transverse	14	38.2 $\pm$ 0.46	12	34.3 $\pm$ 0.63	22	29.8 $\pm$ 0.38	21	26.6 $\pm$ 0.71	20	21.1 $\pm$ 0.51	16	16.1 $\pm$ 0.54
Midthoracic descending anterior	Longitudinal	12	33.5 $\pm$ 0.51	12	28.5 $\pm$ 0.51	22	22.9 $\pm$ 0.46	22	21.4 $\pm$ 0.54	20	17.2 $\pm$ 0.53	17	14.2 $\pm$ 0.43
Midthoracic descending posterior	Longitudinal	11	30.3 $\pm$ 0.75	12	26.8 $\pm$ 0.52	21	19.6 $\pm$ 0.41	22	17.6 $\pm$ 0.53	20	12.7 $\pm$ 0.53	17	11.2 $\pm$ 0.49
Upper abdominal	Transverse	13	36.4 $\pm$ 0.56	11	31.6 $\pm$ 0.61	20	23.7 $\pm$ 0.79	21	19.8 $\pm$ 0.69	20	16.1 $\pm$ 0.57	16	13.0 $\pm$ 0.83
Lower abdominal	Transverse	12	32.6 $\pm$ 0.96	10	27.8 $\pm$ 0.62	16	17.8 $\pm$ 0.62	20	13.8 $\pm$ 0.72	17	11.2 $\pm$ 0.48	11	9.5 $\pm$ 0.55
Pulmonary artery	Transverse	4	33.8	1	29.0	5	27.9	9	21.1	8	24.6	5	19.3

\* Variation in number of observations due to omission of measurements on damaged strips and because the entire aorta was sometimes not available.

\*\* Mean % = amount of retraction divided by maximum stretched length  $\times$  100.

\*\*\* See text for exact localization of areas indicated in this column.

In order to correlate this selective loss of elasticity with the development of intimal lesions, the intimal weight of each portion is tabulated in Table II. In the descending thoracic portions where 2 strips were examined from each area, they were not averaged but included as independent determinations. This accounts for the greater number of observations in these areas. Other variations in the number of observations are due to the exclusion of damaged strips. The weight of the intima shows a progressive accretion with age although the increase is not so constant or so uniform as the decline in elasticity. For example, the mean intimal weight of the segments from the ascending aorta in the seventh decade is less than that for those in the sixth decade, although admittedly the number of observations in these particular groups are relatively few. The lower abdominal portion shows the first significant increase in weight over the other areas. Later the posterior thoracic and upper abdominal portions also develop heavier intimas than elsewhere. The ascending aorta and the anterior thoracic descending portions, as might be expected from casual observations, are covered by the lightest intimal layers. A positive correlation is apparent between the degree of loss of elasticity and the increase in intimal weight. Only in the anterior thoracic portion is there a marked discrepancy. This zone loses elasticity more rapidly in its longitudinal direction than do the transverse thoracic strips, yet its intima remains relatively thin.

The results are graphically recorded in Chart I where the degree of elasticity and the intimal weight are represented by equivalent intensities of shading. The selective loss of elasticity at different levels of the aorta with advancing age shows close coincidence with the increasing weight of the intima except for the one defection already noted.

### *Relation of Intimal Weight to Postmortem Elasticity of the Aorta*

The thickened unyielding intima which develops with advancing age and increasing atheromatous deposits may serve to fix the underlying media and thus limit its flexibility to traction by mechanical interference. The alternative explanation is that the loss of elasticity in these selected areas is due to more rapid degenera-

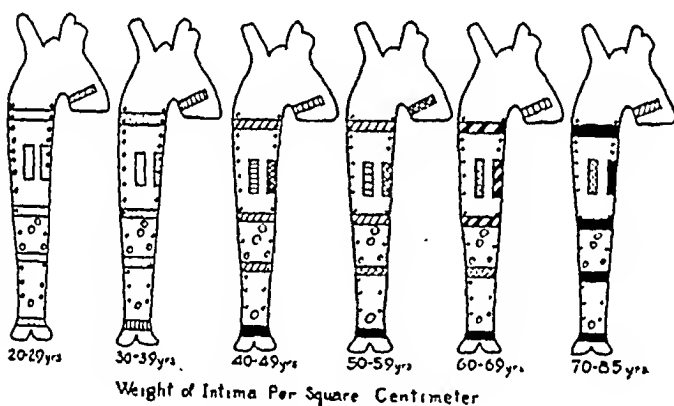
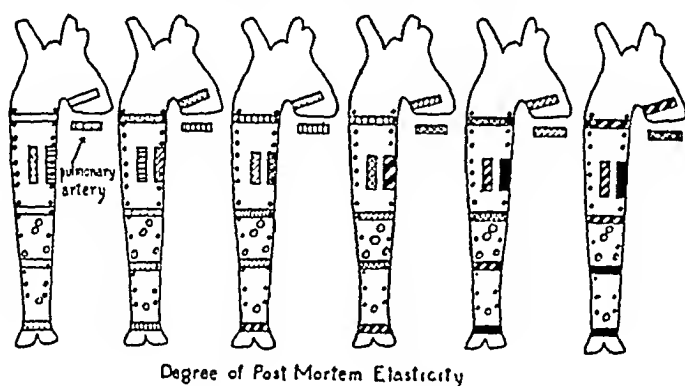
TABLE II

*Relation of Intimal Weight to Age*

Area	Direction	20-29 yrs.		30-39 yrs.		40-49 yrs.		50-59 yrs.		60-69 yrs.		70-85 yrs.	
		No.	Mean gm. per sq. cm.	No.	Mean gm. per sq. cm.	No.	Mean gm. per sq. cm.	No.	Mean gm. per sq. cm.	No.	Mean gm. per sq. cm.	No.	Mean gm. per sq. cm.
Ascending	Transverse	9	0.036	9	0.044	10	0.044	12	0.068	10	0.045	12	0.065
Upper descending thoracic	Transverse	24	0.030	24	0.040	40	0.062	40	0.063	38	0.085	34	0.089
Lower descending thoracic	Transverse	23	0.026	23	0.039	40	0.061	40	0.065	40	0.077	31	0.102
Midthoracic descending anterior	Longitudinal	23	0.023	23	0.029	37	0.053	43	0.053	39	0.066	35	0.068
Midthoracic descending posterior	Longitudinal	20	0.031	23	0.039	36	0.066	44	0.071	35	0.087	33	0.115
Upper abdominal	Transverse	12	0.029	11	0.036	18	0.060	23	0.072	21	0.075	18	0.103
Lower abdominal	Transverse	10	0.038	6	0.049	16	0.083	21	0.088	18	0.089	11	0.140

tion of the elastic fibers, with weakening of the wall, and that the development of intimal plaques is a secondary phenomenon. In order to discover which of these two possible factors is responsi-

CHART I.: Comparison of Intimal Weight and Post Mortem Elasticity at Different Levels in Each Age Group.



% Elasticity.	Wt. of Intima Gms persqcm.
36.0 - 39.0	0.022 - 0.032
31.5 - 35.9	0.033 - 0.043
27.0 - 31.4	0.044 - 0.054
22.5 - 26.9	0.055 - 0.065
18.0 - 22.4	0.066 - 0.076
13.5 - 17.9	0.077 - 0.087
9.0 - 13.4	0.087 + OVER

ble, the following experiments were performed. In the thoracic areas the postmortem elasticity of the media alone, after the intima had been dissected away, was determined in a manner identical with the tests performed on immediately adjacent segments of intact aorta.

The results are shown in Table III, where the postmortem elasticity of the media alone denuded of its intima at various levels in

TABLE III

*Influence of Intima on Postmortem Elasticity of Aorta at Various Ages*

Area and direction	Layer tested	20-29 yrs.		30-39 yrs.		40-49 yrs.		50-59 yrs.		60-69 yrs.		70-82 yrs.	
		No.	Mean %	No.	Mean %	No.	Mean %	No.	Mean %	No.	Mean %	No.	Mean %
Upper descending thoracic	Media * only	13	37.8 $\pm$ 0.54	12	35.4 $\pm$ 0.42	20	31.4 $\pm$ 0.34	21	27.9 $\pm$ 0.55	21	23.7 $\pm$ 0.48	17	19.0 $\pm$ 0.61
Transverse	Intact	15	37.0 $\pm$ 0.46	13	33.7 $\pm$ 0.52	23	29.7 $\pm$ 0.42	21	27.0 $\pm$ 0.58	21	21.5 $\pm$ 0.48	18	16.2 $\pm$ 0.63
Lower descending thoracic	Media only	11	39.0 $\pm$ 0.50	12	36.5 $\pm$ 0.45	19	31.8 $\pm$ 0.24	22	28.8 $\pm$ 0.61	20	24.5 $\pm$ 0.45	16	18.9 $\pm$ 0.63
Transverse	Intact	14	38.2 $\pm$ 0.46	12	34.3 $\pm$ 0.63	22	29.8 $\pm$ 0.38	21	26.6 $\pm$ 0.71	20	21.1 $\pm$ 0.48	16	16.1 $\pm$ 0.54
Midthoracic descending anterior	Media only	9	34.9 $\pm$ 0.67	11	28.9 $\pm$ 0.63	16	25.6 $\pm$ 0.53	22	25.2 $\pm$ 0.51	19	21.5 $\pm$ 0.56	17	18.7 $\pm$ 0.63
Longitudinal	Intact	12	33.5 $\pm$ 0.51	12	28.5 $\pm$ 0.51	22	22.9 $\pm$ 0.46	22	21.4 $\pm$ 0.54	20	17.2 $\pm$ 0.53	17	14.2 $\pm$ 0.43
Midthoracic descending posterior	Media only	9	33.0 $\pm$ 0.72	11	28.3 $\pm$ 1.04	17	24.4 $\pm$ 0.60	21	21.9 $\pm$ 0.60	15	21.0 $\pm$ 0.60	17	17.6 $\pm$ 0.61
Longitudinal	Intact	11	30.3 $\pm$ 0.75	12	26.8 $\pm$ 0.52	21	19.6 $\pm$ 0.41	22	17.6 $\pm$ 0.53	20	12.7 $\pm$ 0.53	17	11.2 $\pm$ 0.49

\* Intima removed from strips by dissection before testing.

the thoracic portion is compared in different age groups with samples of the intact aorta at the same levels. It may be seen that the presence of the intima invariably reduces the degree of retraction of the intact aorta under the conditions of the experiment. This difference is relatively slight in the early decades of life but becomes more marked in older age groups and particularly so in those areas that develop the heaviest intimas. For example, at the posterior thoracic portion in the two oldest age groups the freed media exhibits an elasticity that is 50 to 60 per cent greater than the intact segment. Nevertheless, it is also apparent that the media itself progressively loses its power to retract in older age groups. The reduction in the case of the stripped media is even more uniform throughout life than it is for the intact strip. It is greater in the longitudinal than in the transverse plane. The anterior longitudinal portion shows as rapid a decline as does the posterior longitudinal, so that the greater losses of the latter in the intact condition are largely due to the restraint offered by the thicker accumulations of intimal atheromas which so frequently develop at this site.

The above experiments indicate that the loss of postmortem elasticity which occurs with advancing age is due largely to a failure on the part of elastic fibers to recoil as efficiently after extension, and it is also due in part to the resistance offered by the thickened intima. The possibility still exists that the media, if held immobile beneath intimal plaques, may secondarily lose its ability to stretch and retract perhaps because of disuse. There is some indication that the greatest losses of elasticity occur at points where the vessel is most rigidly attached *in situ*, namely, along the posterior wall and at the bifurcation.

In order to examine more closely for a relation between post-mortem elasticity and intimal thickening, the changes within each age group were explored. In Table IV the segments within each decade are grouped by intimal weight and the elasticity of those covered by the thickest intimas compared with those covered by the thinnest ones. When this is done the number of observations falling into each division are so few that some values show chance variation. This is particularly true when only one observation is recorded. Nevertheless, it may be seen that there is a striking tendency for the mean values of each group within a

TABLE IV

## Relation of Postmortem Elasticity to Intimal Weight of Intact Aorta Within Each Age Group

Age in yrs.	Intimal weight (gm. per sq. cm.)	Ascending aorta		Upper descending thoracic transverse		Lower descending thoracic transverse		Midthoracic descending anterior longitudinal		Midthoracic descending posterior longitudinal		Upper abdominal transverse		Lower abdominal transverse	
		No.	Mean %	No.	Mean %	No.	Mean %	No.	Mean %	No.	Mean %	No.	Mean %	No.	Mean %
20-29	0.010-0.019	1	40.0	1	38.8	4	39.8	3	35.1	1	30.4	5	35.7	1	32.7
	0.020-0.029	3	39.4	9	37.2	2	34.0	5	32.5	6	32.8	1	39.5	3	35.2
	0.030-0.039	3	35.4	4	37.0	3	38.7	2	31.9	1	32.0	2	37.8	3	31.6
	over 0.040	1	35.7	1	33.9	3	38.1	3	33.0	2	34.7	4	36.2	3	32.5
30-39	0.015-0.029	1	32.8	2	34.7	1	35.3	5	28.2	1	21.0	3	33.0	1	27.1
	0.030-0.039	1	35.4	7	34.3	7	33.6	3	28.9	5	27.0	4	33.9	2	30.7
	0.040-0.049	4	35.6	2	31.8	2	34.6	3	28.2	6	28.3	4	29.6	1	29.6
	over 0.050	3	31.8	2	34.5	2	36.0	1	30.4	1	27.6	.	..	3	26.2
40-49	0.019-0.039	6	29.7	6	30.9	6	29.0	8	23.6	3	20.6	4	25.4	2	19.4
	0.040-0.059	2	30.6	8	31.3	12	31.2	11	22.1	11	20.0	6	27.6	3	18.8
	0.060-0.079	1	29.2	6	28.6	1	29.9	1	23.2	4	21.1	2	24.0	1	16.7
	0.080-0.099	.	..	.	..	2	26.8	.	..	1	13.8	3	22.6	4	16.8
50-59	over 0.100	1	32.2	2	26.6	2	27.7	2	23.8	2	16.0	4	18.5	6	17.6
	0.020-0.039	1	16.8	5	29.3	3	26.1	8	22.0	1	20.0	3	23.8	4	16.6
	0.040-0.059	7	27.9	6	24.3	8	26.4	8	21.9	6	17.6	10	20.9	4	18.9
	0.060-0.079	1	29.2	7	27.9	8	27.7	5	19.6	8	19.2	2	21.0	3	10.4
60-69	0.080-0.099	1	25.0	2	24.6	.	..	2	21.2	5	16.2	2	15.6	1	13.8
	over 0.100	2	20.0	1	19.1	2	24.7	.	..	2	13.2	4	15.1	9	11.3
	0.020-0.039	3	22.9	1	28.2	2	24.6	5	18.3	.	..	2	16.3	4	12.8
	0.040-0.059	4	24.6	4	20.6	4	19.4	3	20.2	3	18.1	7	17.9	2	12.5
70-85	0.060-0.079	2	24.1	6	22.1	7	22.2	5	16.1	4	13.8	3	15.4	4	9.4
	0.080-0.099	.	..	5	21.9	4	19.7	3	17.0	6	11.6	3	17.4	1	13.5
	over 0.100	.	..	4	19.0	4	20.7	2	12.6	7	11.3	4	12.6	5	9.5
	0.030-0.059	5	17.4	4	20.1	2	16.0	10	14.6	1	11.1	2	15.7	2	13.5
70-85	0.060-0.079	3	16.5	7	15.5	5	16.5	4	15.1	1	14.8	6	13.2	.	..
	0.080-0.099	2	15.5	2	15.4	5	16.4	2	11.6	4	10.3	1	25.3	2	9.8
	0.100-0.119	.	..	4	14.9	2	14.5	2	13.8	3	13.5	3	11.5	1	10.5
	over 0.120	1	5.6	1	12.1	2	16.4	.	..	7	9.2	4	9.5	5	7.3

given decade to approximate each other. In the groups with heavier intimas there is a slightly lessened degree of elasticity. This is especially the case when the average intimal weight exceeds

TABLE V  
*Relation of Postmortem Elasticity of Media Denuded of Intima to  
Intimal Weight Within Each Age Group*

Age in yrs.	Weight of intima (gm. per sq. cm.)	Upper descending thoracic transverse		Lower descending thoracic transverse		Midthoracic descending anterior longitudinal		Midthoracic descending posterior longitudinal	
		No.	Mean %	No.	Mean %	No.	Mean %	No.	Mean %
20-29	0.010-0.019	.	..	4	39.5	4	34.1	1	30.4
	0.020-0.029	7	38.9	3	39.0	3	33.6	6	32.8
	0.030-0.039	1	36.8	2	38.9	1	35.2	1	32.0
	over 0.040	3	36.8	1	36.7	1	36.8	2	34.7
30-39	0.015-0.029	2	37.0	3	37.7	4	28.5	2	26.0
	0.030-0.039	3	36.5	3	37.0	2	33.3	6	29.3
	0.040-0.049	2	35.8	2	36.9	4	29.0	1	31.1
	over 0.050	4	34.0	2	36.8	.	..	2	25.3
40-49	0.019-0.039	4	30.4	2	30.7	5	26.5	.	..
	0.040-0.059	5	32.9	6	32.8	6	25.2	5	24.3
	0.060-0.079	4	32.5	7	31.4	3	27.0	4	25.3
	0.080-0.099	5	29.2	1	31.7	1	22.2	3	23.3
	over 0.100	1	32.9	2	31.7	1	28.2	2	25.9
50-59	0.020-0.039	4	26.8	1	32.9	3	22.0	1	17.3
	0.040-0.059	5	29.8	8	29.0	10	24.0	8	20.5
	0.060-0.079	5	27.2	5	26.9	3	28.2	5	22.9
	0.080-0.099	2	28.8	2	26.2	2	23.4	2	24.9
	over 0.100	2	26.9	3	29.4	1	30.4	4	23.2
60-69	0.020-0.039	1	20.4	.	..	3	22.6	2	22.2
	0.040-0.059	3	25.2	3	23.9	4	24.3	1	21.2
	0.060-0.079	5	23.8	7	25.5	4	21.8	3	21.6
	0.080-0.099	5	25.2	5	23.3	4	19.2	4	23.4
	over 0.100	5	23.8	2	25.1	3	21.0	3	20.3
70-85	0.030-0.059	2	25.3	.	..	3	21.9	.	..
	0.060-0.079	4	17.5	4	19.3	7	16.8	1	17.3
	0.080-0.099	4	21.3	1	15.3	3	19.6	7	18.6
	0.100-0.119	4	15.6	4	17.4	.	..	3	14.4
	over 0.120	3	17.9	5	20.5	2	20.4	5	17.0

0.100 gm. per sq. cm. As previously noted, at this weight macroscopically appreciable calcification is usually present. It may be concluded then that within a single age group the degree of post-mortem elasticity of the intact aorta declines slightly as the intimal



plaques become more extensive. The variation with intimal weight is minimal when compared with the loss of elasticity that occurs with advancing age so that there is very little overlapping between the various age groups.

When the postmortem elasticity of the media denuded of its intima is examined within each age group with reference to the weight of the detached intima in the same manner, a different result is obtained as shown in Table V. Here the postmortem elasticity of the stripped media is found to be fairly constant at the same level for each age period regardless of the weight of the intima. Such variations as do occur are probably the result of paucity of data, but obviously the media of the segment bearing the heaviest intimas is as elastic as those covered by the thinnest ones in most instances. This would indicate that the decline in elasticity of the intact aorta associated with increasing intimal weight within a single age period, as shown in Table IV, is simply due to the resistance offered by the unyielding intima which becomes more effective as the membrane thickens.

## DISCUSSION

From this elementary approach to the problem a number of significant observations are evident. First of all, the experience of many previous workers is substantiated, namely that there is a progressive reduction in the extensile and retractile properties of the aorta which continues throughout adult life. This reduction develops at a quite uniform rate for a given area and appears to affect all persons to approximately the same extent. The post-mortem elasticity is therefore almost entirely dependent on age. Indeed, the uniformity within a single age group is quite striking, indicating that the elasticity is not readily altered either by disease processes or postmortem changes.

The loss of elasticity, although constant for a given area, varies in different portions. It is more rapid and complete in the longitudinal direction than transversely, along the posterior aspect more than anteriorly, and at the bifurcation rather than at the proximal portions. The only factor that appears to be constant for all regions in which the reduction in elasticity occurs earlier and more rapidly is that they represent portions which by reason of

their attachments during life must have had a limited excursion with each pulse beat. Obviously, a living artery must expand more transversely with each pulsation than longitudinally. The posterior wall and the bifurcation represent areas that are again more firmly united to adjacent bony structures and thus of limited mobility. This suggests either that fixed areas of the aorta lose elasticity simply through relative disuse, or that regenerative or replacement processes are so directed as to maintain the more mobile parts of the aorta in a more elastic condition. Another possibility is that the more firmly attached portions are subject to greater stress and strain. This, however, does not seem very likely and would be difficult to prove or disprove.

There does not appear to be any very direct association between the degree of postmortem elasticity and the extent of intimal lesions. Reduction in elasticity of the media proceeds as rapidly and regularly in those individuals who develop a few small atheromas as in those who develop many large ones. It is true that in the preceding experiments a thickened intima sometimes retarded the mobility of the media but under the lesser degrees of tension to which the aorta is subjected during life it is doubtful whether this degree of limitation is sufficient to hamper the necessary movements of the vessel. The observation that the pulmonary artery undergoes a similar diminution in postmortem elasticity with increasing age, albeit to a somewhat lesser degree, also would seem to divorce this change from an essential relation to the formation of intimal plaques. As is well recognized, this vessel is almost never the seat of intimal lesions unless some disease process interferes seriously with the pulmonary circulation.

The curious localization of the early atheromatous lesions in the areas subject to the greatest losses of elasticity may be entirely unrelated, although it does suggest an indirect but not very obvious connection. If the assumption is made that lipoid material, once it has gained entrance into the intima, is capable of being shifted in position, then a plausible explanation can be offered. There might well be a tendency for it to gravitate to the least elastic and movable parts. A number of attendant circumstances make it seem likely that such an event may transpire. First of all, the earliest microscopically visible accumulations of lipoid material are contained within phagocytes capable of movement. Secondly, there

is a tendency for lipid material to collect about intimal scars, such as the point of closure of the ductus Botalli, at the commissural margins of the aortic valve cusps, or about the orifices of the intercostal arteries. This may be interpreted as evidence that the lipid material has penetrated along tissue spaces in the intima until it reached an impassable barrier.

The usual explanation offered for the focal distribution of intimal lipid deposits is that they occur at points of great stress and strain. There is little positive evidence to support this contention. If anything, one might suspect that the posterior portion of the thoracic aorta would be more protected from strain by reason of its attachment to bone. Similarly, one would also expect that the anterior, more movable regions, where the large abdominal vessels arise, would be subjected to greater stress and strain, yet these areas are less often involved by early lipid deposits. Another more striking example is afforded by the mitral valve leaflets. Here obviously the greatest point of stress and strain must be at the contact or distal edges of the leaflets. Yet lipid deposits accumulate not infrequently only at the basal, less movable and more protected portions. The ease with which the intima may be detached from the media after death is an indication that these two layers are but loosely amplexant and suggests planes of dissection along which lipid may be transferred. If lipid material within the intima is capable of spreading, an attractive explanation for the development of focal accumulations is apparent. The lipid may well gain entrance initially in minute amounts over large areas of the surface and only later come to lodge in discrete circumscribed deposits.

The reduction in elasticity with age is probably chiefly dependent on changes in the elastic fibers. Neither the smooth muscle nor the connective tissue can play a very fundamental role, both by reason of the physical properties of each of these two tissues and because of their architectural arrangements within the media. Inert smooth muscle strips, as represented by the muscularis of the intestine, will, it is true, undergo marked elongation when subject to traction but will fail to retract on release to more than 10 per cent of the maximum stretched length. Connective tissue, as exemplified by chordae tendineae and tendons, offers great resistance to traction and can only be stretched to a length about

6 per cent greater than when released. Inasmuch as even in senile persons the aorta still possesses as much retractility as either of these two types of tissue, it seems fairly reasonable to assume that any greater degree of retraction is dependent on the properties of the elastic fibers. Architecturally, the framework of the smooth muscle is anchored to the delicate reticulum sheaths that enclose the elastic fibers. The effects of traction depend therefore on the ability of the elastic fibers to elongate.

The most striking alteration that the elastic fibers undergo with advancing age is a loss of undulation. Loss of elasticity as well as dilatation of the vessel as a whole is probably reflected by this failure on the part of the elastic fibers to recoil on relaxation as efficiently as formerly. Ranke<sup>20</sup> has shown that the elastic fibers have properties analogous to fine metal coils and that stretching is accomplished merely by the straightening of the coils under tension and not by any yielding or thinning of the substance of the fiber itself. In vessels distended and fixed in formalin at pressures exceeding the greatest ever experienced during life, the fibers are almost but not quite straight, whereas with decreasing pressures the wavy contours return. An opposite point of view has been maintained, most recently by Andrus,<sup>21</sup> that the elastic fibers are rubbery in nature and stretching is accomplished by their thinning out. The degree of undulation is from this point of view merely slack caused by excessive muscular contraction and true stretching does not begin until the elastic fibers are straight.

Some of the confusion over this relatively simple point may have been caused by the fact that 10 per cent formalin fixation for 24 hours does not destroy completely, although it greatly reduces, the elastic properties of elastic tissue. Arteries fixed in a distended state in this manner may show some retraction when freed from pressure that might alter the histological appearance. Support for Ranke's concept can be easily obtained by stretching 1 of 2 adjacent strips of aorta with a weight of 1500 gm., attaching the extended strip to a firm support to prevent retraction and fixing both the stretched and relaxed strips in Zenker's fluid. This fixative completely abolishes the elastic properties of the vessel wall. As seen in the microphotographs, taken at the same magnification, of sections prepared with Weigert's elastic tissue stain, maximum stretching is accomplished without any thinning of the

elastic fibers which remain the same in diameter as in the undistended strip. After death at least, the elasticity of elastic fibers is not comparable to that of fine rubber bands.

Since a fine metal coil resists compression as well as extension and has a tendency to return to a fixed shape when relaxed, it is easy to understand why an aorta freed of its *in situ* attachments tends to assume a constant shape and size dependent on the ability of the elastic fibers to recoil. This force is presumably great enough to overcome any resistance on the part of the smooth muscle and connective tissue.

### SUMMARY AND CONCLUSIONS

The postmortem elasticity of the aorta as measured by the ability of excised portions to retract following extension is a constant and durable property which varies only with age. In the young adult the elasticity is approximately equal in all areas and in both the transverse and longitudinal directions. With advancing age there is progressive loss of elasticity which varies in degree in different areas but proceeds at a constant rate for a given area and affects all persons to approximately the same extent. This reduction in elasticity is associated with a failure on the part of the individual elastic fibers to recoil and assume a wavy appearance on relaxation and is commensurate with the enlargement of the vessel as a whole.

Reduction in elasticity occurs first and proceeds more rapidly in those areas that are most rigidly attached *in situ* and in the longitudinal plane. Such areas are presumably capable of less movement with each pulsation during life.

The development of intimal plaques is not directly related to this loss of elasticity. A greatly thickened intima can restrict the elasticity of the media to some extent. Loss of elasticity of the media proceeds as rapidly in those subjects who develop slight intimal atheromas as in those who exhibit profound changes. The earliest and largest intimal accumulations of lipid material appear in those areas that are subject to the earliest and most marked loss of elasticity. An hypothesis is advanced to account for this coincidence. This is based on the assumption that lipid material does not necessarily remain at its point of entrance into the intima

but may be influenced by the movements of the vessel during life to lodge in the least elastic and least movable portions. Loss of elasticity with age occurs constantly in an artery, the pulmonary one, not usually the seat of intimal plaques.

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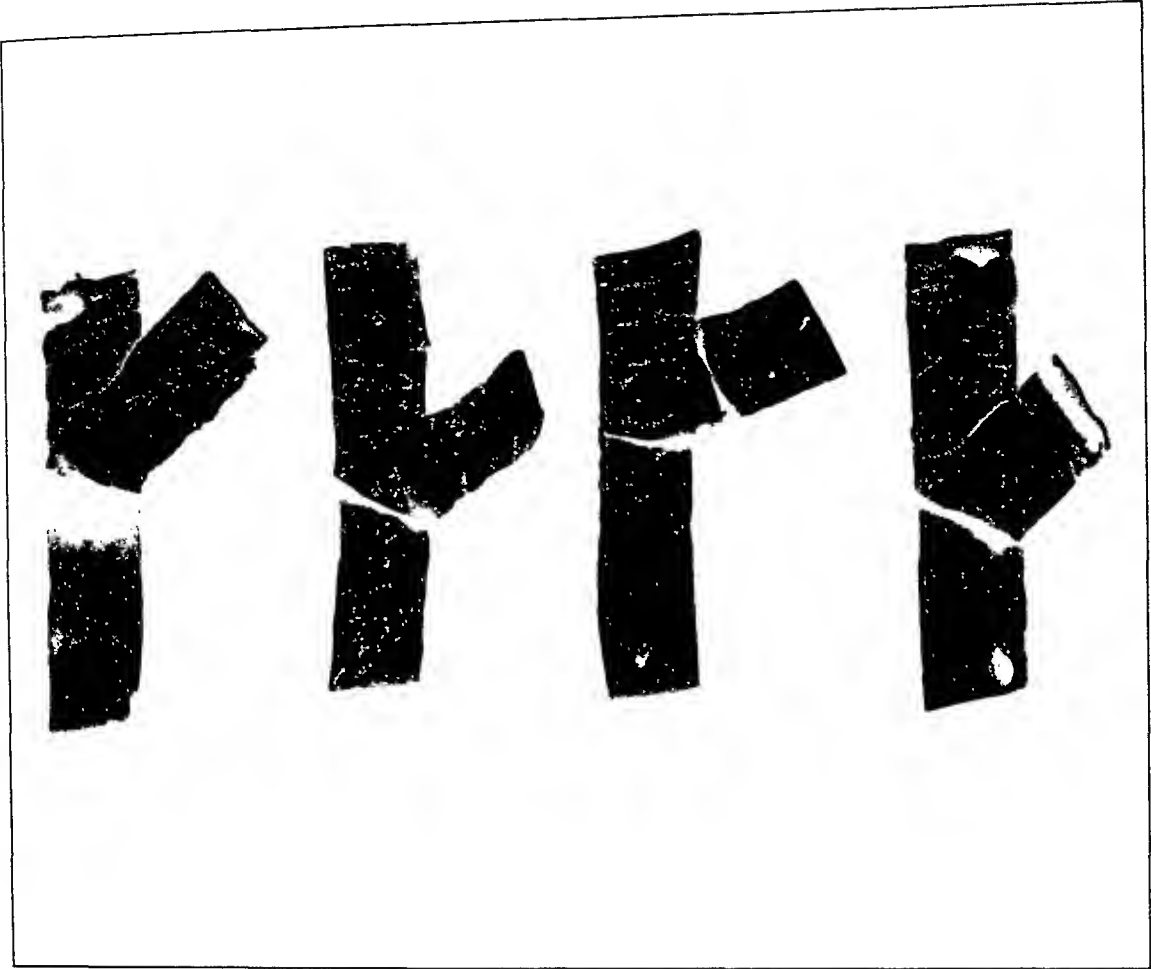
## DESCRIPTION OF PLATES

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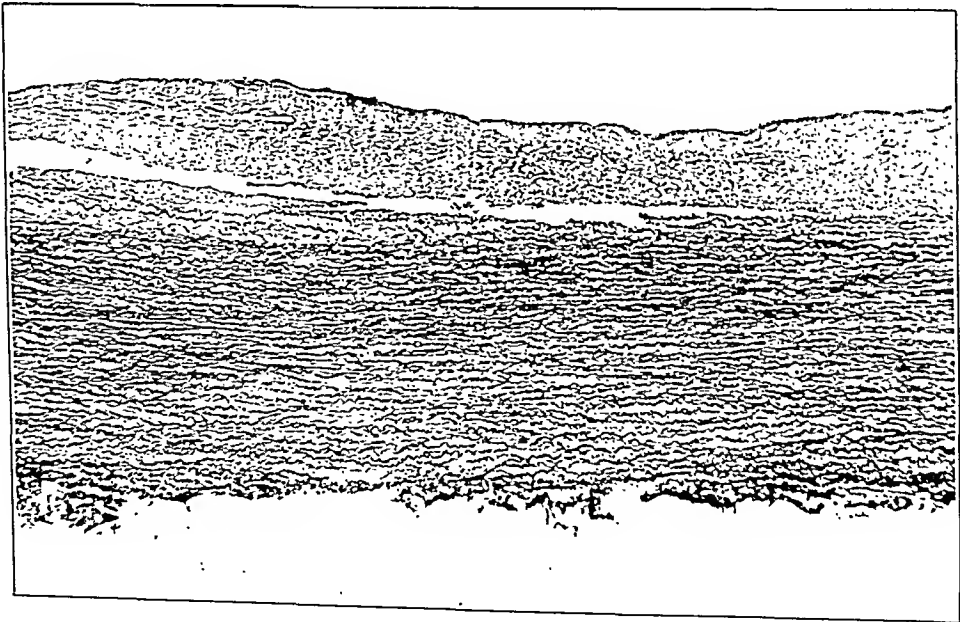
### PLATE 112

FIG. 1. Strips of aorta with the intimal layers partly freed and reflected backward. The intima separates as a thin membrane including the bulk of intimal plaques. The exposed medial surface is smooth and intact.

FIG. 2. Microphotograph of strip of aorta with intima detached from media. The clear space represents the line of separation which lies deep within the intima and leaves the underlying media relatively undamaged. Weigert's elastic tissue stain.  $\times 50$ .



1



2

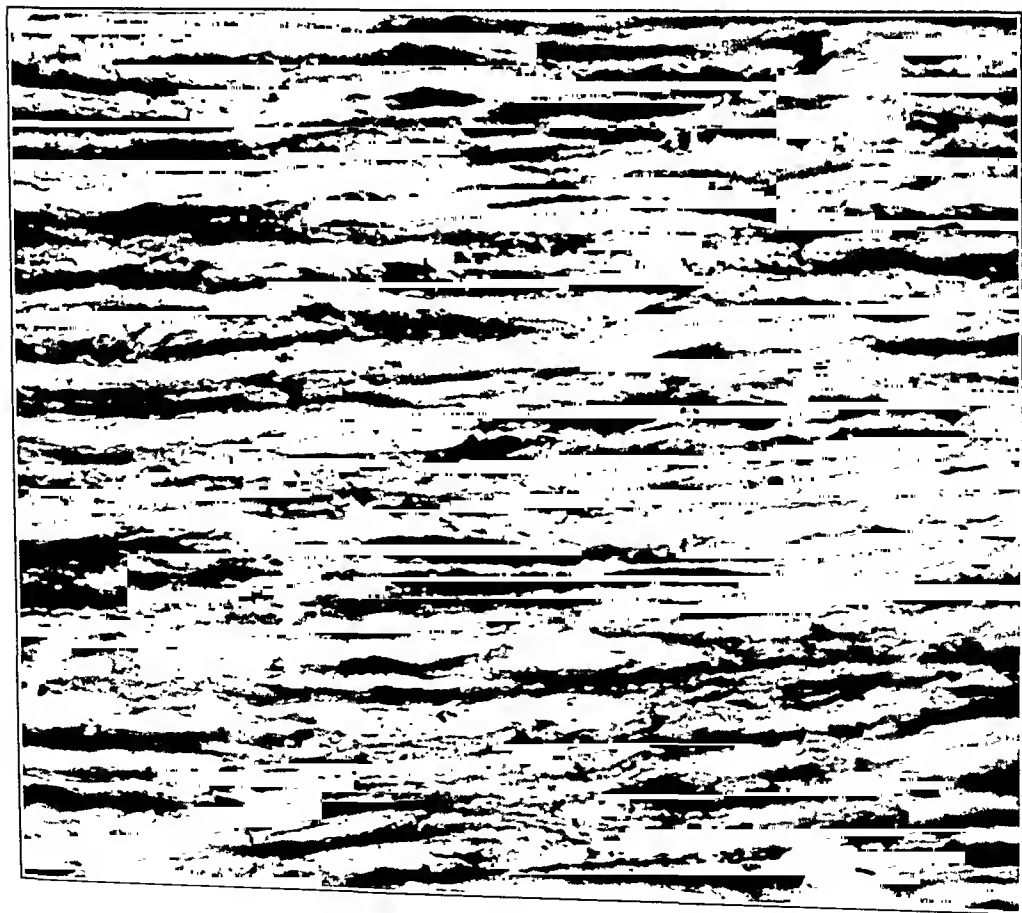


### PLATE 113

FIGS. 3 and 4. Microphotographs taken at same magnification of two adjacent strips of aorta. The upper one was fixed in the usual manner; the lower one while stretched with 1500 gm. of traction. In the unextended strip the elastic lamellae show their usual wavy contours. In the stretched one they have become straightened, lie closely together and are parallel to each other, but the average thickness of the fibers is unchanged. Weigert's elastic tissue stain.  $\times 300$ .



3



4



# LOCALIZED CONGENITAL DEFECTS OF THE CARDIAC INTERVENTRICULAR SEPTUM \*

## A STUDY OF THREE CASES

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Phylogenetically and pathologically cardiac malformations have long excited a lively interest but only comparatively recently have cardiologists given serious consideration to the matter of establishing as certainly as possible the nature of such conditions during life. That accuracy of diagnosis in congenital heart disease can be improved is shown by the figures of McGinn,<sup>1</sup> who reports that by means of careful and systematic study the incidence of correct or suggested diagnoses was raised from a previous percentage of 48 to 85 per cent. As evidence that pathologists are becoming increasingly alert in searching for cardiac anomalies, McGinn also points out that among 4100 autopsies falling in the period between 1895 and 1920 congenital heart lesions had an incidence of 0.6 per cent as compared with 1.2 per cent among 3400 cases examined during the 1921-1935 interval, an increase felt to be the direct result of a greater interest in the growing accumulation of information about malformations of the heart.

Among the rarer developmental abnormalities of the heart are localized defects of the interventricular septum, not at the base of the heart just anterior to the pars membranacea, which is the common site, but elsewhere in the septum or multiple. In only 5 of the 1000 cases analyzed by Abbott<sup>2</sup> is this condition listed as primary, and it occurred as a complication of other anomalies in only twelve additional hearts. By way of contrast it may be mentioned that the same statistics show 257 cases of defects at the pars membranacea. From the standpoint of comparative anatomy Abbott and Shanly<sup>3</sup> have stated that the homologue of septal

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defects of the type under consideration exists in the python (*P. murulus*), in which there exists normally a communication low in the septum between the dorsal and ventral ventricles.

In the small group of muscular septal imperfections the case described by Weiss<sup>4</sup> stands in a class by itself. His subject was a male who attained the mature age of 79 years without cardiac symptoms, aside from a purely terminal cyanosis. Auscultation revealed a loud, harsh, systolic murmur, most intense over the ensiform but still audible over the entire cardia; the aortic and pulmonic sounds were not accentuated. The heart borders were not easily defined but did not seem to be increased. Edema was absent. The electrocardiogram displayed a rate of 84, normal atrioventricular bundle conduction, impaired and delayed right branch bundle conduction, and evidence of right ventricular preponderance and of myocardial degeneration. The unusual position of the intense thrill and the harsh quality of the murmur, together with the peculiar cyanosis, suggested the possibility of a latent congenital lesion such as a septal defect, and this was the probable clinical diagnosis although the location of the communication could not be established.

Postmortem examination of the heart disclosed a circular defect, 2 cm. in diameter, located in the posterior half of the interventricular septum about midway between the apex of the left ventricle and the mitral ring and communicating with the right ventricle near the apex by means of an aneurysmal pouch and four or five fenestrations. The edge of the hole on the left side of the septum was fibrous and smooth but directly above it was a roughened spot covered by a mural thrombus. Neither of the ventricular walls was thicker than normal. The features distinguishing the lesion as congenital were the location, size and smooth edges of the defect, together with the natural openings between the trabeculae carneae on the right ventricular aspect.

Among nearly 8000 autopsies on individuals of all ages we have encountered congenital imperfections in the muscular division of the interventricular septum three times, always in children of 12 years or less. In two of these the only important anomaly is a single point of communication between the ventricles. One of these we believe to be unparalleled in the literature for the reason that while the muscle is interrupted the actual opening is so

small that little if any shunting of blood could have taken place. In the third heart there are two points of interventricular communication in an otherwise essentially normally developed organ.

### CASE REPORTS

CASE 1: (No. 9546). No information concerning this 12 year old white boy is available other than that he dropped dead while attending church services. The autopsy was ordered by the coroner.

#### *Description of Heart*

Aside from the inconsequential congenital fusion of two costal cartilages, the only congenital condition is in the heart, which is dilated and pushes the left lung toward the thoracic wall. The heart and aorta as far as the midthoracic level weigh 165 gm. The organ is rotated so that the anterior presenting surface is formed by the right chambers alone. The right atrium in particular is dilated. The foramen ovale is closed; the thebesian valve persists. There exists a communication (Fig. 1) between the ventricles through the muscular part of the septum immediately adjoining the junction of the septum with the anterior wall of the heart. The ostium measures 1 by 0.8 cm. on the left ventricular side, 1 by 0.6 cm. on the right side, and courses obliquely caudalward from left to right. The cephalad margin of the opening lies 1.5 cm. below the junction of the muscular and the membranous parts of the septum. The latter is quite intact. Along the anterior margin of the defect are two ridge-like trabeculae carneae; elsewhere the border is smooth and even. The left and right ventricular endocardium fully covers the defect and while thicker and more whitish than elsewhere is free from any signs of vegetations or thrombi. The ventricles appear to be of equal size, although by actual measurement the wall of the right proves to be 0.8 cm. in thickness at the apex and 1.3 cm. at the base, as compared to 0.7 and 1.1 cm. for the left at the same points. The posterior and left anterior leaflets of the pulmonary valve are fenestrated, as are also the anterior and left posterior cusps of the aortic valve, although to a lesser degree. There are three right coronary ostia, measuring 3, 1.5, and less than 0.5 mm., respectively. The left coronary artery ostium has a diameter of 3.5 mm. The

distribution of the coronary vessels is the most frequent of the normal varieties. The tricuspid and mitral valves are unchanged save for three oval-shaped atheromatous plaques in the anterior leaflet of the latter valve. Several transversely directed fatty streaks occur in the aortic intima at the level of the valve commissures. The main pulmonary artery is similarly involved, except that the plaques extend onward into the left primary branch. The pulmonary valve has a circumference of 6.3 cm., while the aortic ring measures 4.5 cm. The great vessels enter and leave the heart in the normal manner. The ductus arteriosus, although patent, is only 1 mm. in diameter.

*Anatomical Diagnoses:* Congenital malformations of the heart: (a) isolated defect of muscular portion of interventricular septum, (b) fenestrated aortic and pulmonic valve cusps, and (c) multiple (3) right coronary ostia; persistent patent ductus arteriosus; persistent thebesian valve; hypertrophy of right ventricle; pronounced dilatation of right atrium; moderate dilatation of left ventricle; atheroma of anterior mitral valve leaflet, intima of ascending aorta, main pulmonary artery and its left branch; generalized acute passive hyperemia; and congenital fusion of two costal cartilages.

*Alteration of the Circulation:* The venous blood entering the right side of the heart was propelled by the hypertrophied right ventricle into the pulmonary bed in the usual way. Some oxygenated blood from the left ventricle no doubt was ejected into the right ventricle through the imperfect septum during systole providing the normally higher left ventricular pressure existed. In support of this concept is the preponderance of the pulmonary valve ring over the aortic and the greater thickness of the right ventricular wall. While the ductus arteriosus was patent it appears to be too small to have had any functional significance.

CASE 2: (No. 538-10-35): The subject was a boy, 6 years of age, with an abnormally long bleeding and clotting time who died from hemorrhage following tonsillectomy and adenoidectomy. There is no history of any cardiac symptoms during life, the heart lesion being discovered in the course of a routine autopsy.

### *Description of Heart*

The right side of the heart is much dilated and is largely responsible for the transverse diameter of 13 cm. The whole organ

weighs 110 gm. It is normally formed save for a minute fenestration of the right posterior aortic valve leaflet, the presence of three right coronary artery ostia, two of which are very small, and the interruption of the muscle of the interventricular septum. On viewing the septal aspect of the left ventricle one's attention is drawn to a pit-like area surrounded by a rim of pearly white endocardium (Fig. 2), situated about midway between the apex and the aortic ring, 5 mm. from the junction of the septum with the anterior wall and 9 mm. from the posterior wall. The trabeculae carneae on the septal side end abruptly at the inferior margin of the lesion. The raised whitish rim about the crater-like depression measures 6 by 4 mm. A sagittal cut carried directly through the apparent stoma reveals a complete interruption of the muscle of the septum at this level by what appears at first glance to be a solid cord of whitish tissue joining with the endocardium of either ventricle. However, on more careful inspection there is discovered at the center of the apparent cord a minute opening 0.5 to 1 mm. in diameter having a smooth and glistening lining but devoid of any blood. The stoma of the defect on the right ventricular side is slit-like and much less prominent than that on the left side. The exact point of attachment of the thickened band of endocardium projecting outward into the right ventricle (Fig. 2) is unknown, owing to the fact that it was divided in opening the right ventricle at autopsy before the presence of the abnormality was discovered. When the cut margins are reapproximated, however, the band comes to lie very near the base of the papillary muscle of the anterior tricuspid valve leaflet. The right terminus of the defect is 5 mm. from the point where the anterior wall of the right ventricle joins with the interventricular septum. On the left side of the septum the interrupted myocardium is rounded off along either side of the fibrous endocardial tube, but on the right side only the inferior border is bevelled. Two yellowish atheromatous patches occur in the anterior mitral leaflet and a number of similar streaks involve the aortic intima within the sinuses of Valsalva and just superior to them. Both the foramen ovale and the ductus arteriosus are obliterated.

*Anatomical Diagnoses:* Congenital malformations of the heart: (a) defect of septum interventriculorum through its muscular portion, (b) fenestrated right posterior aortic valve leaflet, and (c)



multiple (3) right coronary ostia; focal fibrosis of endocardium in immediate vicinity of defect; atheroma of aorta and anterior mitral valve cusp; and acute dilatation of the right heart chambers.

*Alteration of the Circulation:* Neither the clinical history, physical examination nor the pathological observations indicate that any significant amount of shunting of blood took place through this minute anomalous communication. The pronounced dilatation of the right heart was acute and can be accounted for by the fatal postoperative hemorrhage.

CASE 3: (No. 207-4-32). This 7 year old girl collapsed and died suddenly after being struck over the manubrium sterni by a batted indoor baseball. She was roller skating at the time of the accident. Dr. Roy A. Payne, a neighbor of the family, had been asked rather casually to examine her about a year previously, and finding some enlargement of the heart to the left, together with a double murmur over the mitral area, requested the parents to bring the child to his office for a complete study, but this was not done and he did not see the girl professionally again. He had noted that she was able to play like other children of her age but seemed to fatigue easily.

### *Description of Heart*

At autopsy there was found a zone of hemorrhage in the soft tissues over the manubrium sterni at the point of impact of the ball. All organs except the heart were normally developed.

The heart weighs 150 gm. Anteriorly the right ventricular wall has a thickness of 1 cm. and at the base posteriorly measures 1.4 cm., as compared to 1 cm. and 1.3 cm. for the left ventricle at corresponding points. The myocardium displays no macroscopic changes. The tricuspid valve has a circumference of 9.5 cm., including a point of cleavage 1.6 cm. in extent situated between the inferior and medial leaflets. Otherwise this valve is unchanged. The membranous part of the interventricular septum is lacking and in its place is an oval opening (Fig. 3) 1.8 by 2.3 cm. in size with the longer diameter directed somewhat anteroposteriorly. The superior border of the hole is formed by the smooth and sharp edged interatrial septum with parts of the inferior and medial cusp of the tricuspid valve forming the upper anterior and posterior margins. All other parts of the boundary consist of the septal myocardium, covered by normal appearing endocardium. Where the muscular part of the interventricular septum joins the posterior wall of the heart is a second defect situated between two large trabeculae carneae. On the right side of the

septum the opening measures 0.7 by 1.4 cm., the long diameter being directed superior-inferiorly and the lesser one anteroposteriorly. On the left ventricular aspect the opening is smaller, measuring 0.6 by 0.8 cm. The endocardium covering the perforation is unchanged. Crossing the inferior border on the left side is a large bundle of muscle, obviously one of the trabeculae carneae, which passes cephalad to end at the inferior border of the defect at the site of the absent membranous septum. Posterior to this band and at the same level as the opening in the muscular division of the partition is a third fenestration 2 by 2 mm. in size which communicates with the main opening. The mitral valve circumference is 8 cm. The posterior leaflet is shorter than its mate and possesses two distinct papillary muscles, one quite small, the other of average normal size. The anterior cusp has a thick and rolled margin, and like the other leaflet has two very stout papillary muscles. All chordae tendineae attached to this cusp are short, thick and pearly white. No verrucae or other forms of vegetations occur on either of the mitral cusps. The anterior mitral leaflet is on a level with the opening through the membranous septum and prevents the latter from being seen from the left ventricular side. Viewed from the right ventricle the mitral leaflet forms a curtain-like background along the upper half of the defect. The myocardium is everywhere unaltered. The aortic valve ring has a circumference of 3.2 cm., the pulmonic a circumference of 5.5 cm. In the intima of the ascending aorta and the main pulmonary artery are a few fatty streaks. The coronary arteries are normally distributed. The foramen ovale and ductus arteriosus are closed. There are no abnormalities of development or of position on the part of any of the great vessels leaving and entering the heart.

*Anatomical Diagnoses:* Multiple congenital lesions of the heart: (a) defective interventricular septum — (1) pars membranacea and (2) pars muscularis, (b) cleavage of inferior and medial leaflets of the tricuspid valve, (c) supernumerary mitral papillary muscles; fibrosis and shortening of mitral valve leaflets and chordae tendineae; hypertrophy of both cardiac ventricles; pronounced dilatation of right heart chambers; early atheroma of aorta and main pulmonary artery; generalized acute passive hyperemia; and recent contusion of soft tissues overlying the sternum.

*Alteration of the Circulation:* All available evidence points to an arteriovenous shunting via the two points of communication between the ventricles. The diversion of arterial blood from the arterial to the venous circuit could have been very considerable because of the large size of the openings. Clinical data are meager and we have no means of knowing whether or not there was a terminal reverse of blood flow. Shock due to trauma seems to have been the precipitating factor in the acute right heart failure, as is suggested by the great distention of the right side of the heart observed at autopsy.

### SUMMARY AND CONCLUSIONS

Three new cases of cardiac malformation consisting of one or more communicating openings between the ventricles without any other anomalies of serious nature are added to the very small group already recorded in the literature.

All occurred in children between the ages of 6 and 12 years, two being males, the other a female.

Two display a single defect in the muscular division of the interventricular septum; the third shows in addition to this a defective pars membranacea, placing it in the clinical category of "maladie de Roger."

Cases 1 and 3 belong in the acyanotic group of congenital heart disease with transient or terminal reversal of blood flow. Case 2 is most remarkable, not because of any cardiac symptoms, for any appreciable shunting in either direction was impossible, but on account of its dissimilarity to the others herein described or in the literature. It is interesting, and from the standpoint of correct classification most important, that although the opening is a minute one it nevertheless possesses a complete lining of endocardium and for this reason may properly be termed a true congenital interventricular septal defect.

Evidence of arteriovenous shunting during life is plainly written in the hypertrophied right ventricular walls of Cases 1 and 3. While lamentably little is known about the lives of these children it would appear that the strain on the hearts in Cases 1 and 3 initiated by the septal imperfections was quite grave, for the 12 year old boy died suddenly while sitting in church and the 7 year

old girl succumbed to an injury in itself hardly sufficient to cause death. In the boy it is possible that emotional activity may have precipitated the acute heart failure; in the girl it is quite conceivable that trauma to the chest induced shock which could not be borne by the unbalanced heart. The heart murmur heard in Case 3, described by the examiner as a double sound over the mitral area, is of doubtful significance for typically the murmur produced by interventricular septal fenestra is holosystolic and very harsh.

All subjects escaped the most serious and frequently occurring complication of congenital heart disease (endocarditis) with the possible exception of the girl in Case 3, in whom the fibrosis of the mitral valve suggests healed rheumatic disease. Against this is the absence of histological changes attributable to rheumatic fever in the fibrotic valve or the myocardium. A more plausible explanation for the thickening is long continued trauma and strain on the valve due to its proximity to the defect in the pars membranacea where with each systole there could have been a splitting of the column of blood, thus simultaneously exposing both sides of the valve to wear and tear.

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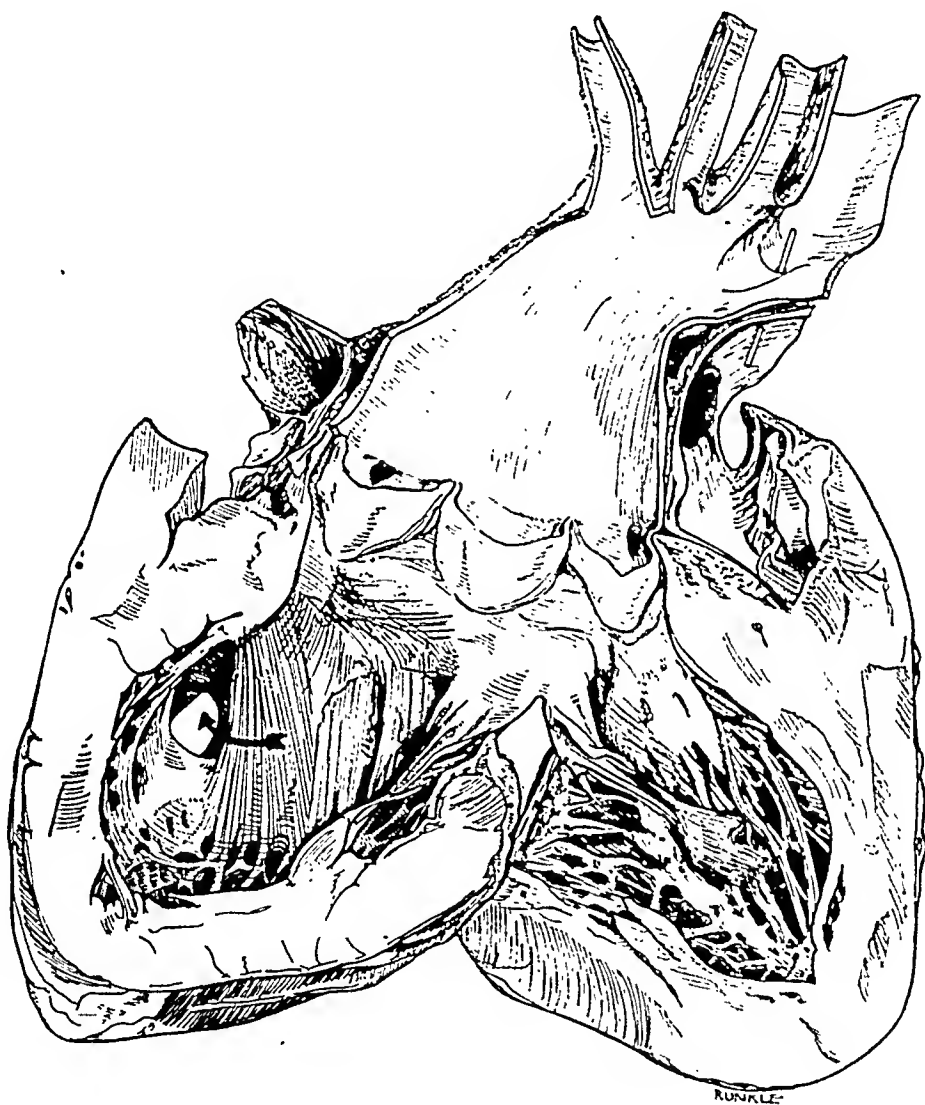
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## DESCRIPTION OF PLATES

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### PLATE 114

FIG. 1. Drawing of left side of heart from Case 1, showing position of inter-ventricular septal defect, two of the three right coronary ostia, and the patent ductus arteriosus through which a bristle has been passed.



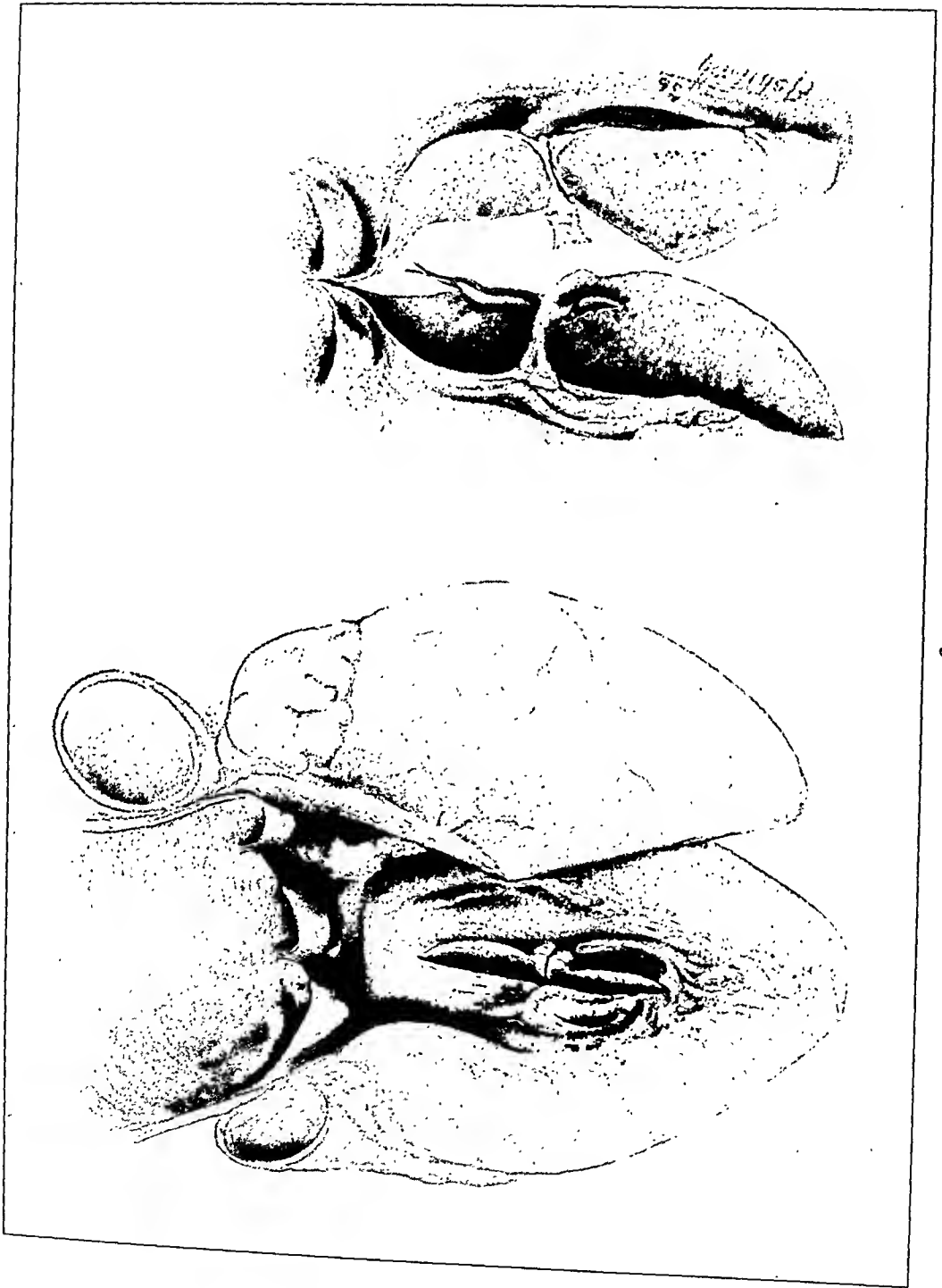
I

Mason and Hunter

Defects of Cardiac Interventricular Septum

PLATE 115

FIG. 2. Two views of the endocardial tube from Case 2 filling the imperfection in the muscular segment of the interventricular septum. A portion of the channel has been exposed by means of a shallow incision in the left aspect of the septum. The entire course of the opening is shown in the smaller drawing in which the sagittal cut has been completed and the margins everted. Note the pearly character of the endocardium about the left terminus of the defect.



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Defects of Cardiac Interventricular Septum



PLATE 116

FIG. 3. In this drawing of the heart reported in Case 3 the right chambers have been opened and widely everted in order to bring the two communications into full view. Interrupting the tricuspid valve is the larger opening at the position of the pars membranacea; in the background beyond this is the margin of the anterior mitral cusp and one of its chordae tendineae, accounting for the small fenestration. The ovoid defect in the muscular division of the septum is separated from that in the membranous septum by a considerable amount of muscle.





# HYPERPLASIA AND REGENERATION OF THE MYOCARDIUM IN INFANTS AND IN CHILDREN \*

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The purpose of this paper is twofold: first, to point out that in cardiac hypertrophy of infants there may be an active proliferation of myocardial elements in addition to growth by enlargement of the individual muscle fibers; and secondly, to prove that during childhood heart muscle fibers may regenerate following severe injury. Both of these observations are in contradiction to current opinion. In textbooks and journals it is stated that the increase in size of the heart in cases of cardiac hypertrophy is due to an increase in size of the individual muscle fibers, and is not the result of a multiplication of these. Furthermore, it is almost axiomatic that the one and only form of myocardial healing subsequent to necrosis of heart muscle fibers is by the proliferation of connective tissue with ultimate repair by fibrosis. The former hypothesis in all likelihood holds true for the enlarged hearts of adults irrespective of cause, and is based primarily on the following two reasonably well established facts. First, careful measurements of the muscle fibers of hypertrophied hearts of adults have shown that an increase in size and weight of the heart may be explained mathematically on the basis of an increase in size of the individual myocardial fibers. Secondly, patient search throughout the myocardium in such cases of cardiac hypertrophy has failed to reveal any positive evidence, in the form of mitoses, of true myocardial proliferation. This frequently recorded absence of mitoses is also the most important single fact on which the statement that heart muscle fibers can not regenerate is based.

## MYOCARDIAL HYPERPLASIA

It is rather difficult to determine at what age an infant's heart may be considered to have acquired its full complement of muscle fibers. It is generally believed that at the time of birth of a full-

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term child, the heart, though less than a tenth of its adult size, is completely formed. The rarity with which a single mitotic figure is found in average sized hearts, even at birth and during the first month of life, lends weight to this viewpoint and strongly suggests that an active proliferation of muscle elements under ordinary conditions ceases quite early. The increase in size of the infant's heart from this period to adult life, estimated by a progressive gain in weight, may be readily accounted for by an increase in size of the individual muscle fibers. The chance finding of an isolated mitosis in a heart muscle fiber of an average sized heart of a newborn infant is probably nothing more than a sign of a normal but somewhat delayed physiological process. In contrast to this the presence of many mitoses in heart muscle fibers of enlarged hearts in infants of 1 and 2 years of age, though probably nothing more than an acceleration of this normal growing process, may be justifiably regarded as a type of hyperplasia. This possibility of an increased proliferation of heart muscle fibers has been entirely overlooked as a factor in the histiogenesis of cardiac hypertrophy of infants.

A study of this problem of the normal and pathological growth of heart muscle elements was prompted by the finding of an unusually large number of mitoses in the myocardial fibers of an enlarged heart of a 6 months old child. This child died in uremia, the result of an anomalous development of the mucosa in both ureters, in which valve-like folds were formed causing complete intermittent urinary obstruction. The heart of this child was well formed. It weighed 60 gm., which is just twice the average weight for a child of this age and size. It showed particularly an enlarged left ventricle with an increase in thickness of the left ventricular wall. In a careful histological examination of two other enlarged hearts from infants of 12 and 20 months of age respectively — comprising a case of so-called primary idiopathic hypertrophy, as well as a case of cardiac hypertrophy secondary to coarctation of the aorta — it has also been possible to demonstrate the presence in heart muscle fibers of mitoses in all stages of nuclear division. The debatable interpretation of this finding as to whether it should be considered as true hyperplasia or merely a somewhat more active stage in the natural histiogenesis of the myocardium would seem to be of secondary importance. More important is

the indisputable fact that during this early age growth of the myocardium by an active proliferation of muscle elements may and does take place in cardiac hypertrophy of infancy.

The histological appearance of the myocardium in cases of cardiac hypertrophy in infancy varies. In the case of congenital idiopathic hypertrophy the increase in size of the individual fibers was scarcely perceptible. The fibers showed normal branching and well developed myofibrils. Mitoses were seen in the muscle fibers in the peripheral portion of the myocardium. In the 2 cases of secondary hypertrophy associated with coarctation of the aorta and diminished urinary elimination, the muscle fibers were distinctly larger than normal and the myofibrils were increased in number. This true hypertrophy was not found absolutely uniformly throughout the ventricular wall, for at times small delicate fibers and large thick fibers were seen lying side by side. In these 2 cases, in addition to an increase in size of the muscle fibers, mitoses were also found. They were most numerous toward the periphery and toward the tip of the left ventricle. Both cases showed normal myocardial branching. The fibers were regularly arranged and transverse striations throughout the myofibrils were everywhere seen. At this age, no transverse lines of segmentation, intercalated discs, have yet appeared.

The division of the nucleus occurs at the center of the fiber (Fig. 1). All stages of mitotic division are recognizable, including the single spireme, the monaster, diaster and double spireme (Figs. 2, 3 and 4). At the site of nuclear division one centrosome is seen at the end of each spindle (Fig. 5). The spindle is large and long search may be necessary to find one entirely intact (Fig. 6); serial sections are helpful in distinguishing questionable mitoses. The chromatin material appears as clumps of granules and short rods and stains intensely with hematoxylin. This chromatin material in a dividing nucleus may be scattered rather irregularly over the spindle (Figs. 2 and 3). The cytoplasm at the site of nuclear division is comparatively clear and is free of myofibrils (Fig. 6). Usually myofibrils showing transverse striations are seen at each side of the spindle, while just beyond each end of the dividing nucleus the cytoplasm becomes more intensely stained and delicate transverse striations, best demonstrated by Mallory's phosphotungstic acid hematoxylin stain, are visible in the myoplasm be-

fore longitudinal fibrils in this area are clearly recognizable. This differentiation of these transverse striations in the myoplasm appears to represent the early growth and development of the myofibril in the proximity of the dividing nucleus.

### MYOCARDIAL REGENERATION

During the preparation of this paper, which originally was planned to include only the problem of myocardial proliferation in cardiac hypertrophy in infancy, my attention was called by Dr. F. B. Mallory to an autopsy that was performed under his supervision in 1916. A boy, 6 years of age, had died several days after the onset of diphtheria. The most striking finding at the postmortem was the presence of a dilated heart showing a yellowish discoloration of the myocardium. A careful histological examination of the heart muscle showed severe degenerative changes with necrosis of the muscle fibers. This was associated with edema of the stroma and a moderate exudate of endothelial leukocytes and lymphocytes. There was an early proliferation of connective tissue of the myocardium. Most interesting, however, was the presence of isolated mitotic figures in heart muscle fibers bordering these zones of destruction (Fig. 7). This case appears to be the first to have been recognized as showing positive evidence, in the form of mitoses, of true myocardial regeneration. This is apparently a most unusual finding and yet its presence is absolute proof that regeneration may occur. Such observations as this point to the necessity of a more careful histological study of this condition in children. Moreover, it probably explains, in part at least, the frequent absence of myocardial scarring in hearts of children who, months or years earlier, had suffered severe myocardial injury. Finally, it supports from a histological standpoint the recognized need of prolonged rest during the period of recovery and repair following severe myocardial injury; and at the same time it offers the possibility and hope of a *restitutio ad integrum* subsequent to necrosis of myocardial fibers in children.

### REVIEW OF LITERATURE

To review the rather extensive literature dealing with the subject of the growth and regeneration of heart muscle fibers it is necessary to begin with a number of papers that appeared about

the middle of the last century. At that time the development and use of the microscope was limited and the methods used in the preparation of sections for histological study were comparatively poor. For these reasons it is difficult to evaluate the conclusions drawn in these early papers, for often these were based much more on theory than fact. Goldenberg in 1886 reviewed the literature on this subject since 1850, discussing the earlier papers of Kölliker,<sup>1</sup> Foerster,<sup>2</sup> Vogel,<sup>3</sup> von Rokitansky,<sup>4</sup> Friedreich,<sup>5</sup> Paget,<sup>6</sup> and Rindfleisch,<sup>7</sup> a group of investigators who considered the hyperplasia of muscle fibers in cardiac hypertrophy merely as an accepted fact. It is of interest that Lebert<sup>8</sup> as early as 1857 pointed out that there was no histological basis for this point of view. Goldenberg<sup>9</sup> believed primarily in the hypertrophy of the individual muscle fibers as the best explanation for myocardial hypertrophy. He said, however, that hyperplasia by longitudinal splitting could occur. Considerable importance was attached to a paper appearing in 1875 by Zielonko,<sup>10</sup> a student of Virchow. This investigator, working on the hearts of frogs and rabbits, came to the conclusion that the hypertrophy of the heart was not merely the result of an enlargement of individual muscle fibers, but probably the outcome of cellular hyperplasia as well. This hypothesis was transferred at once from animal to man to explain the myocardial hypertrophy of the human heart, but it lacked proof. No proliferation had been seen and the idea was based on a second and equally questionable hypothesis, "that hypertrophies are greatest in the young and middle ages, because during this period physiological development is still going on, and growing stimuli are at hand." Tangl,<sup>11</sup> after making careful measurements on heart muscle fibers, and after failing to find a single mitosis in a study of hearts showing cardiac hypertrophy, came to the conclusion that hypertrophy of the heart was to be explained entirely by a hypertrophy of the muscle elements. Ziegler<sup>12</sup> adopted a similar view but added that it is possible that the number of muscle cells are increased as well. Wideröe<sup>13</sup> also could find no mitoses and believed that in the absence of mitoses one could reasonably conclude that in cases of cardiac hypertrophy one was dealing simply with a hypertrophy of muscle fibers. Adami and Nicholls,<sup>14</sup> in speaking of hypertrophy of the myocardium, say, "Microscopically the muscle fibres are increased in



thickness and probably in number," but found no absolute evidence of true proliferation.

The idea of myocardial proliferation gained some support from a group of investigators who studied the repair process following myocarditis. They believed they saw signs of active regeneration following severe injury. Saltykow<sup>15</sup> in 1908 made the statement that in both acute and chronic myocarditis the muscle elements play a very important rôle in regeneration, with the result that there is a formation of new myocardial fibers. No mitoses were found in any of these cases. This paper was supported by Heller,<sup>10</sup> who made many drawings of muscle fibers, showing variations in size and shape of the nuclei, but again no mention was made of the presence of mitoses. Warthin,<sup>17</sup> as recently as 1924, believed he saw signs of muscle regeneration in 3 of 15 cases of diphtheritic myocarditis and said, "Near the necrotic or degenerated portion of the heart muscle, the nuclei show great variety in size and form. They increase in length and show evidence of longitudinal splitting in every possible stage of division. The living muscle substance bordering the injured area also undergoes longitudinal splitting into muscle bands containing nuclei. These bands grow into the perimysial tubes filling these up, replacing cell detritus and connecting with the living muscle on the other side of the defect. Muscle bands without nuclei, but accompanied by myoplastic nuclei, also extend into the tubes occupied by the dead muscle substance. These bands lie at the periphery of the tube, and in some cases appear to form a hollow cylinder enclosing the remains of dead muscle substance." No mention is made here by Warthin of even a single mitotic figure.

The more recent papers of Collier<sup>18</sup> and Karsner, Saphir and Todd<sup>19</sup> conclusively pointed out that in myocardial hypertrophy there is an increase in width and length of the sarcomere, or in other words, a hypertrophy of the muscle fiber. These latter investigators repeating the work of Goldenberg and Tangl made careful measurements of the muscle fibers of both hypertrophied and atrophied hearts of adults. They considered that the number of nuclei per field in an atrophic heart is increased, but a most careful search failed to disclose a single mitotic figure. They added, however, "It is possible that since the hearts were obtained postmortem, any mitotic figures present might have been com-

pleted." Kaufmann,<sup>20</sup> Aschoff,<sup>21</sup> and Mönckeberg<sup>22</sup> also stated emphatically that in myocardial hypertrophy the fibers increase in volume with an increase in the number of myofibrils and a proportionate increase in the size of the nucleus. There is no proliferation with an increase in the number of fibers, and mitotic division of the nuclei is absent. MacCallum,<sup>23</sup> and Boyd<sup>24</sup> in their texts likewise subscribe to this view. Mention of the possible proliferation of myocardial fibers in infancy was made by Rössle<sup>25</sup> who said, "With age the fibers increase in thickness, the number of bundles increases, fibers increase in length and muscle elements increase after birth." The possibility of regeneration of muscle elements was supported by Mallory<sup>26</sup> who stated that in very young children there is a general hyperplasia of muscle elements following injury.

### SUMMARY

Evidence is presented in the form of mitotic division of the nuclei of heart muscle fibers to indicate first, that in cardiac hypertrophy of infants a proliferation of heart muscle fibers can take place, and secondly, that in severe myocardial injury in children, regeneration of myocardial elements can occur.

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## DESCRIPTION OF PLATES

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### PLATE 117

FIG. 1. Section of myocardium showing three mitotic figures in heart muscle fibers. The fibers vary in width and show branching. Capillaries are dilated. There is an increase in fluid in the intercellular spaces. Myocardial hyperplasia, congestion and edema.  $\times 330$ .

FIG. 2. One of the three mitotic figures from Fig. 1 at greater magnification. This nucleus is cut slightly on the bias; the small circle is just below one pole of the spindle, and the chromatin material appearing as rods and granules is clustered about the spindle. No definite arrangement of the chromatin can be distinguished. The cytoplasm immediately adjacent to the spindle is comparatively clear, rather finely granular and free of both transverse and longitudinal striation. Myofibrils showing transverse striation are visible at both sides of the dividing nucleus.  $\times 740$ .

FIG. 3. One of the three mitoses seen in Fig. 1 at higher magnification. A spindle is not visible. The chromatin appears as rods and granules unevenly distributed, forming in the center a rather coarse clump. The cytoplasm at the site of nuclear division is comparatively clear. At each side myofibrils showing cross striations are visible.  $\times 740$ .



PLATE 118

FIG. 4. One of the three mitoses seen in Fig. 1, more highly magnified. The mitotic figure is incomplete. No spindle can be distinguished. The chromatin is forming an incomplete circle, appearing as short rods and granules. A comparatively clear zone completely surrounds the dividing nucleus. Myofibrils showing transverse striations are distinguishable laterally and above and below.  $\times 740$ .

FIG. 5. Section of myocardium, showing a mitotic division of a nucleus of a heart muscle fiber. A complete spindle with centrosomes is visible. The chromatin is arranged at the center, forming two rather even rows of coarse granules and rods (monaster). The cytoplasm at the site of nuclear division is clear and very finely granular. Laterally a delicate, non-striated membrane bounds the muscle fiber. Above and below the nucleus the cytoplasm is denser, stains more deeply, and transverse striations appear before individual myofibrils are recognizable.  $\times 740$ .

FIG. 6. Section of myocardium, showing a mitotic figure in nucleus of heart muscle fiber. The entire spindle is recognizable, including one centrosome. The chromatin appearing as clumps of granules and short rods is gathered about the center of the spindle to form an equatorial plate. There is a clear zone about the nucleus. Myofibrils showing transverse striations are seen at each side of the nucleus. Above and below the nucleus the cytoplasm is more intensely stained and transverse striations are visible in the myoplasm before longitudinal fibrils are clearly seen.  $\times 740$ .



4



5

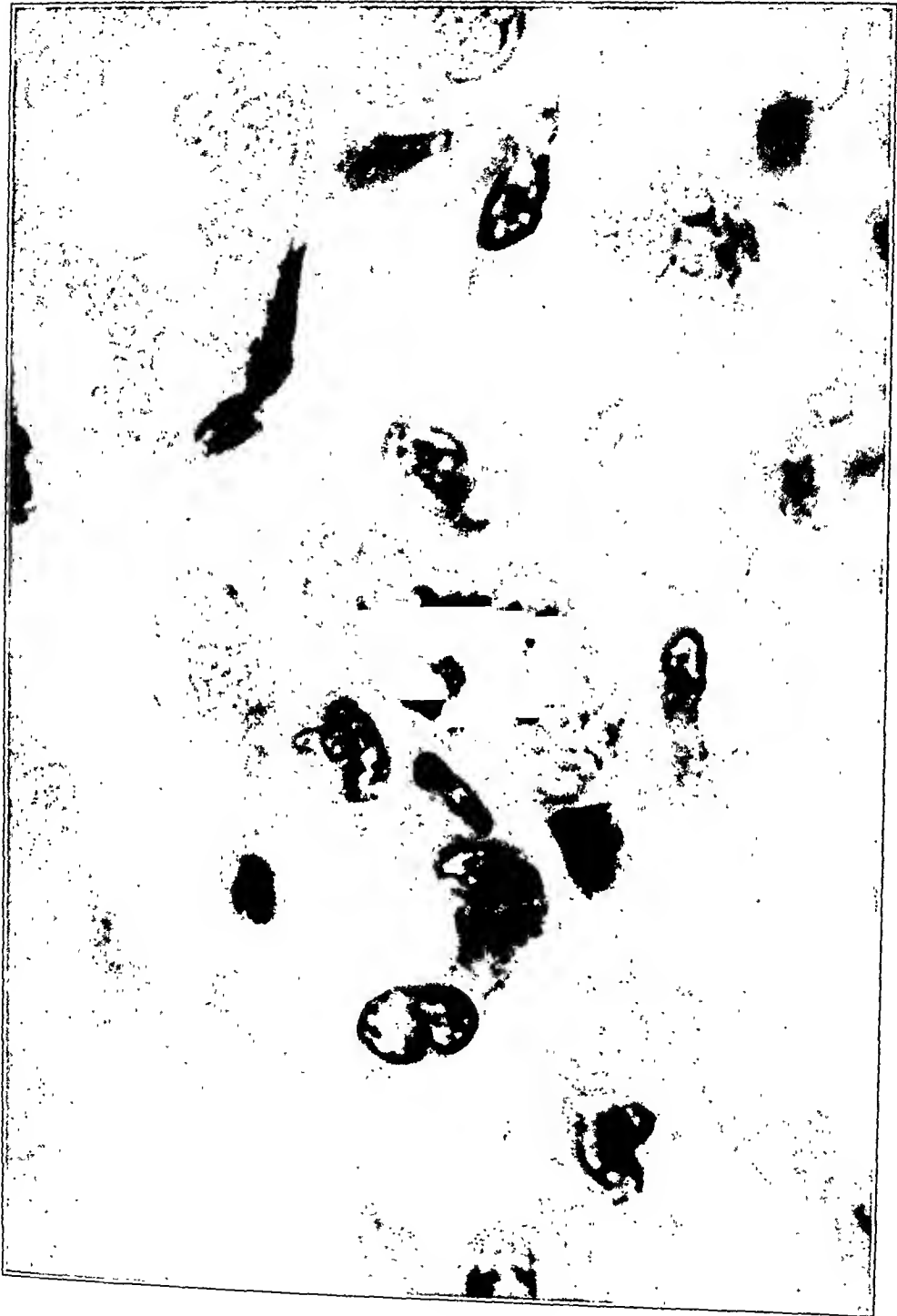


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PLATE 119

FIG. 7. Section of myocardium from a 6 year old boy who died from diphtheria. In the center of the field is a segment of muscle fiber showing a nucleus in an early stage of mitotic division. The chromatin appears as coarse clumps and short rods and is gathered to form an irregular spireme. At this stage no spindle is recognizable. Capillaries are dilated and the tissue shows both a fluid and cellular inflammatory exudate. (BCH-A-16-66, Dr. Mallory.)  $\times 2000$ .



7

MacMahon

Hyperplasia and Regeneration of Myocardium



# MORPHOLOGICAL CHANGES IN THE SUPERIOR VENA CAVA AND RIGHT AURICLE IN RHEUMATIC HEART DISEASE \*

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According to the records of this laboratory, in a few cases of rheumatic heart disease changes were observed in the superior vena cava. These changes were localized at the junction of the vein and the right auricle, and were characterized by yellowish plaques in the intima and media with little or no inflammatory reaction. It was thought desirable to study systematically a larger group of cases, in order to ascertain the nature of the morphological changes, the frequency of their incidence, and whether they occurred only in the presence of rheumatic heart disease.

Seventy-three cases showing definite rheumatic endocarditis were selected for the study. Hearts with superimposed bacterial endocarditis were not included, in order to avoid the possibility of confusion with inflammatory changes of non-rheumatic origin. The ages of the patients ranged from 3 to 66 years. The majority of the patients died from the effects of rheumatic heart disease. In a few cases, individuals dying from other causes, the rheumatic carditis was clinically unsuspected. A control series of 15 hearts without any evidence of rheumatic disease was studied for comparison. In 10 of these the heart was normal, while in 5 there was hypertrophy secondary to chronic nephritis or arteriosclerosis. The ages of the patients in the control group ranged from 4 to 65 years.

Blocks were taken from the superior vena cava with the adjacent portion of the right auricle, and were fixed in Zenker's fluid without the addition of acetic acid or formalin. The sections were stained with hematoxylin and eosin, and by Weigert's elastic tissue stain combined with van Gieson's picro-fuchsin.

The lesions found in the cardiovascular system are shown in

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Table I. In 16 of the hearts there were definite lesions in the superior vena cava. Among these 16 the changes were interpreted as rheumatic in 8, whereas the nature of the lesions in the other 8 was difficult to evaluate. In both groups the lesions were found in the endocardium at the junction of the superior vena cava, sometimes more towards the auricular side, at other times more to the venous side. In 57 of the cases there were no demonstrable changes in this region.

The macroscopic changes in the first group of 8 cases interpreted as being rheumatic in nature were as follows: In 4 cases the endocardium was slightly wrinkled and thickened over areas varying from 2 to 5 mm. in diameter. These plaques were yellowish in color and the surface was dull and slightly irregular. In 2 cases tiny, dull grayish yellow, slightly elevated verrucae 1 to 2 mm. in diameter were present. In 2 cases no gross lesions were evident and the lesions were found only microscopically (Table II).

The histological alterations were as follows: The intima, media and adventitia were thickened, partly because of increase in connective tissue, and partly because of swelling of the collagenous fibers. In addition there was slight infiltration of cells. Many of these cells were large with a faintly blue cytoplasm in hematoxylin-eosin preparations; their nuclei were vesicular and oval, and some contained a large stellate nucleolus. These cells had the usual characteristics of the Aschoff cells. In the outer third of the media a few of these cells were diffusely scattered. In the adventitia they formed typical Aschoff nodules which in some instances extended into the media (Fig. 1). In addition to these larger cells there were a moderate number of lymphocytes, plasma cells, a few polymorphonuclear leukocytes and cells with distorted elongate nuclei (Figs. 2 and 5). The long axis of these nuclei was usually directed perpendicular to the surface. This cellular infiltration was particularly increased in the outer third of the media, but in some instances the superficial layers of the intima were involved. Mainly lymphocytes and plasma cells were found in this region. In some cases the cellular infiltration and thickening of the intima and media were inconspicuous and the main finding was the Aschoff nodules in the adventitia (Fig. 3). As a rule there was an increased diffuse infiltration of lymphocytes in the adventitia.

In elastic tissue preparations the elastic fibers in the intima were seen to be spread apart and fragmented at the site of the cellular infiltrations. The internal elastic lamella was often interrupted and distorted, or was split into a fine meshwork of elastic fibers. The elastic fibers were always arranged parallel to the surface. In some areas loss of smooth muscle elements and increase of connective tissue were noted in the media.

The inflammatory changes described above have thus some of the features of the auricular rheumatic endocarditis described by MacCallum,<sup>1, 2</sup> and by VonGlahn,<sup>3</sup> and have also certain characteristics in common with rheumatic aortitis (Pappenheimer and VonGlahn<sup>4</sup>). However, the lesions are not so extensive in this region as in the left auricle and the aorta.

In the other group of 8 hearts the alterations were different. The intima was definitely thickened with one or more distinct yellowish plaques measuring 4 to 5 mm. in greatest diameter. The surface of the plaques was smooth and glistening. In 1 heart there was no distinct plaque but the intima was more diffusely thickened over an area measuring 2 by 1.5 cm. (Table II). The thickening of the intima was due to collagenous tissue (Fig. 4). No inflammatory reaction was noted within the plaques, but in the deeper portion of the media and the adventitia, in some instances, lymphocytic infiltrations were found; in others no cellular accumulations were present. There were many fine elastic fibrils in the thickened intima, which lay parallel to the surface. In most instances the media was thinned out beneath the plaque and showed a definite loss of elastic fibers and smooth muscle.

It is difficult to evaluate the exact nature of these plaques. In one instance Aschoff nodules were found in the adventitia beneath the plaques, but these do not justify any conclusion as to the nature of the changes found in the plaque. In the cases with lymphocytic infiltrations of the adventitia and deeper portion of the media the reaction was not necessarily of rheumatic origin. It is possible that the plaques represent the end stage of the acute rheumatic lesions described above. This, however, cannot be proved as no intermediate stage between the plaques and the acute rheumatic lesions were found. The supposition that the plaques represent healed lesions is supported by some of the data given in Table II. The patients with the rheumatic lesions were younger

than those in the other group; 6 were under 20 years, whereas only 2 were under 20 years of age in the group with the plaques. Furthermore, 3 of the patients with the rheumatic lesions had a very short rheumatic history and all of them showed either clinically or anatomically an acute exacerbation of their rheumatic condition before death. In the other group with the plaques all except 1 had had longstanding rheumatic heart disease with increasing symptoms of cardiac insufficiency. On the other hand, all these 8 patients had had chronic passive congestion, and it is therefore possible that the plaques were only sclerotic in nature, belonging to the type of changes called phlebosclerosis. The plaques did not contain any fat, so they cannot be regarded as atherosclerotic. Our control group includes too few cases to permit the conclusion that these plaques are found only in rheumatic heart disease.

One feature of interest is revealed in Table II, namely that most of the patients with the rheumatic lesion in the superior vena cava were females. No satisfactory explanation can be given for this.

The acute rheumatic lesions found in 8 cases may be regarded as a special localization of an acute rheumatic process. Lesions of rheumatic nature in the superior vena cava have been observed by Klinge,<sup>5</sup> but they were localized to the more peripheral parts of the vessel. In his paper on rheumatic auriculitis VonGlahn mentions only 1 instance of rheumatic auriculitis in the right auricle. In that case a chain of tiny verrucae was present along the anterior margin of the fossa ovalis. Some of the cases studied in this series showed very small verrucae, both in the neighborhood of the fossa ovalis and in the region of the superior vena cava. Apparently the rheumatic lesions show some preference for these regions of the right auricle.

The incidence of this rheumatic lesion is, as shown in Table I, greater than the valvulitis of the pulmonic valve, rheumatic aortitis or arteritis.

In the group of 15 control hearts no lesions similar to those described above were found.

#### SUMMARY AND CONCLUSIONS

In a study of the superior vena cava at its entrance into the right auricle rheumatic lesions were found in 8 out of 73 cases of

TABLE I  
*Localization of Lesions in 73 Cases of Rheumatic Heart Disease*

<i>Localization of Lesions in 73 Cases of Rheumatic Heart Disease</i>											
Rheumatic lesions found at routine autopsy											
Total No.	Mitral valve	Aortic valve	Tricuspid valve	Pulmonary valve	Left auricle	Myocardium	Pericardium	Aortitis	Arteritis	Superior vena cava	
										Rheumatic	Unspecified
73	69	46	34	7	29	51	26	4	6	8	8



TABLE II

*Sex and Age Distribution, Duration of Illness and Type of Gross Lesions in Superior Vena Cava Found in 16 Cases of Rheumatic Heart Disease*

Autopsy No.	Sex	Age	History of rheumatism	Gross lesion
5989 Babies Hosp.	F	9 yrs.	6 mos.	Small wrinkled areas
6338 Babies Hosp.	F	11	6 mos.	Small verrucae
6389 Babies Hosp.	F	7	1 yr.	Small wrinkled areas
6456 Babies Hosp.	F	12	Attacks since age of 2 yrs.	Small wrinkled areas
11432 Presbyterian Hosp.	F	38	1 attack in childhood	Two verrucae
11524 Presbyterian Hosp.	F	17	Growing pains in childhood, now 6 mos. history	Small wrinkled area
11562 Presbyterian Hosp.	F	12	6 attacks since age of 5 yrs.	None
11580 Presbyterian Hosp.	M	35	Undiagnosed	None
6087 Babies Hosp.	M	8	3 attacks in 4 yrs.	Plaques
6187 Babies Hosp.	F	9	Second attack in 6 mos.	Plaques
11117 Presbyterian Hosp.	M	27	1 attack 11 yrs. ago	Plaques
11251 Presbyterian Hosp.	F	32	4 attacks in 8 yrs.	Plaques
11390 Presbyterian Hosp.	M	54	1 attack in childhood	Plaques
11536 Presbyterian Hosp.	F	36	1 attack 12 yrs. ago	Plaques
11543 Presbyterian Hosp.	F	35	Attacks 23 and 7 yrs. ago	Wrinkled yellowish thickening of intima
11697 Presbyterian Hosp.	F	36	6 attacks in 9 yrs.	Plaques

rheumatic carditis. The inflammatory reactions in the media and the adventitia were similar to those seen in the auriculitis and aortitis of rheumatic origin.

In 8 additional cases hyaline plaques were found. It could not be decided whether these plaques represented the final and healed stage of the acute rheumatic lesions, or whether they were sclerotic in nature and due to longstanding heart disease.

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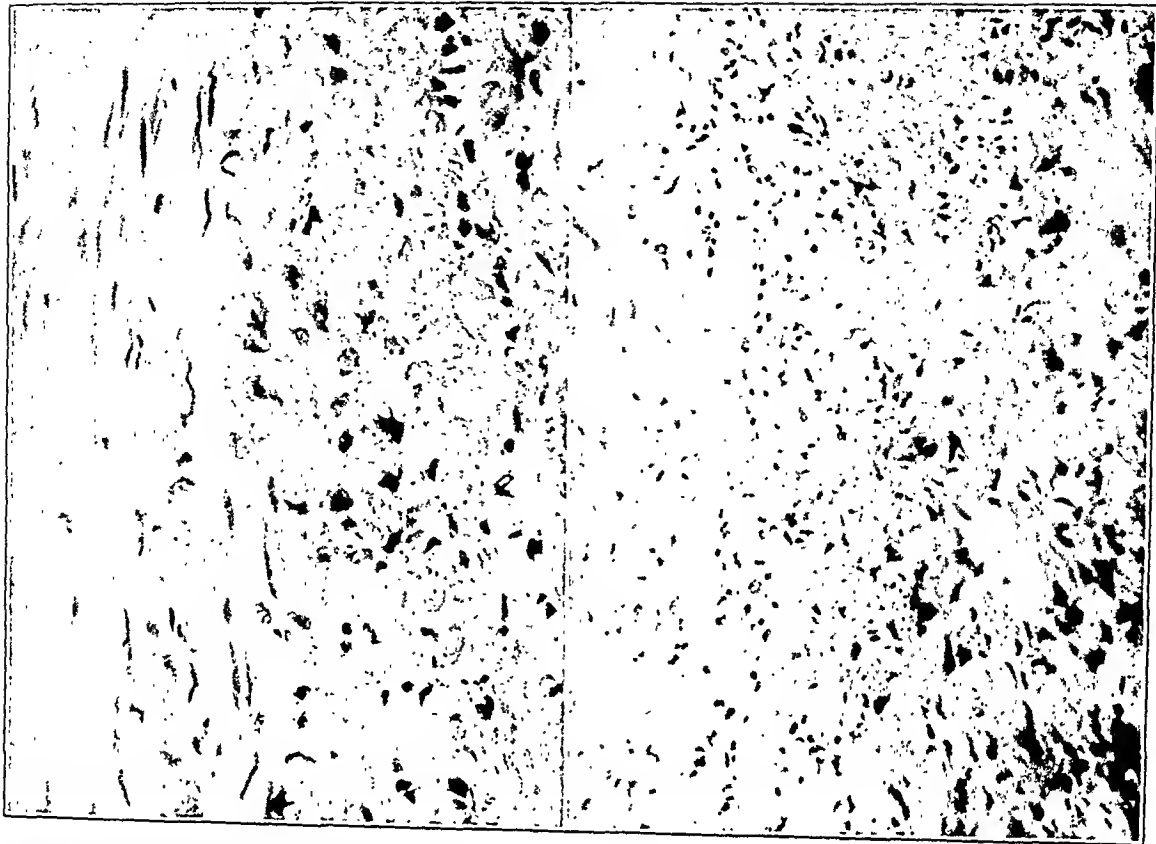
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## DESCRIPTION OF PLATES

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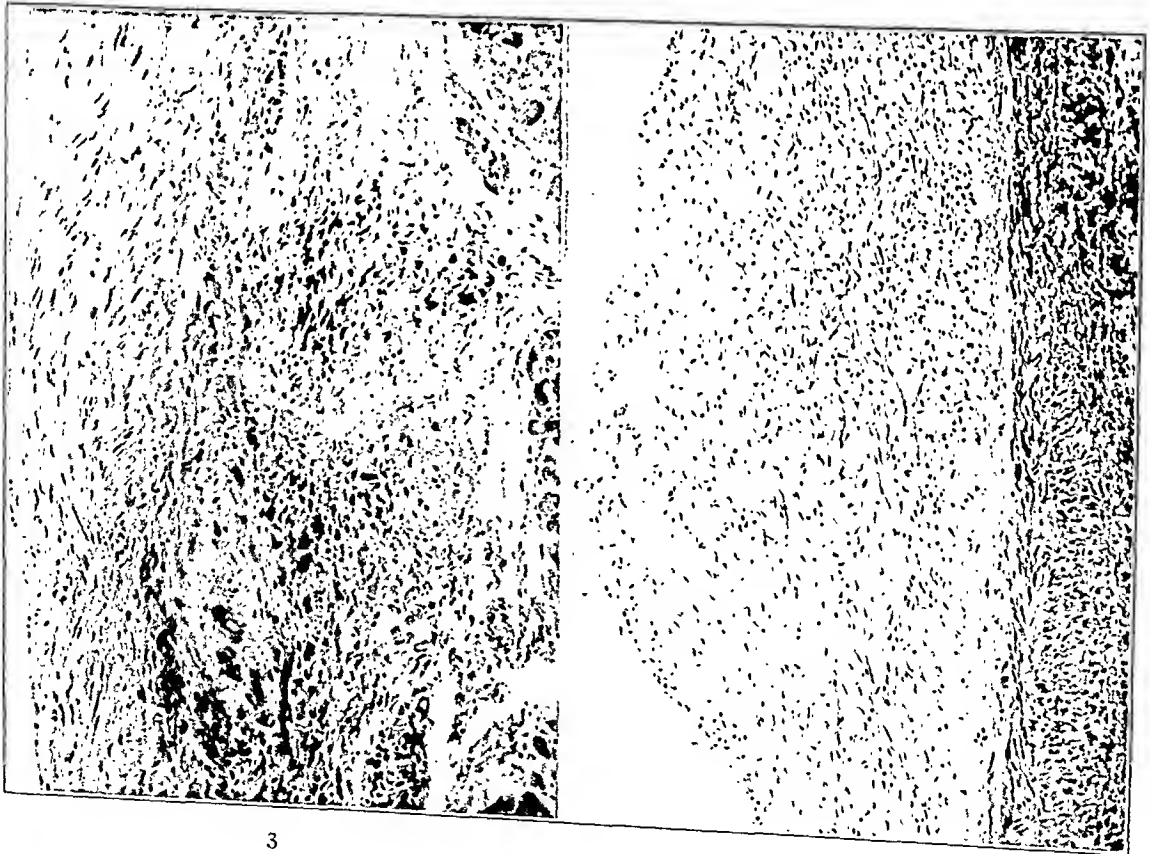
### PLATE 120

- FIG. 1. Autopsy No. 5989. Acute rheumatic endocarditis of right auricle at the entrance of the superior vena cava. Aschoff nodule in adventitia extending into the deeper portion of media. Hematoxylin-eosin stain.
- FIG. 2. Autopsy No. 5989. Acute rheumatic endocarditis of right auricle at the entrance of the superior vena cava. Thickening of media with cellular infiltration, lymphocytes, plasma cells, cells with elongate, distorted nuclei. Hematoxylin-eosin stain.
- FIG. 3. Autopsy No. 11580. Acute rheumatic endocarditis of right auricle at the entrance of the superior vena cava. Aschoff nodules in adventitia. Hematoxylin-eosin stain.
- FIG. 4. Autopsy No. 11117. Hyaline plaque in the superior vena cava at its entrance into the right auricle. Hematoxylin-eosin stain.



1

2



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4

Waller

Morphological Changes in Rheumatic Heart Disease

PLATE 121

FIG. 5. Autopsy No. 6338. Acute lesion of the superior vena cava at its entrance into right auricle. Thickening of media and adventitia and cellular infiltration. Hematoxylin-eosin stain.



5

Waler

Morphological Changes in Rheumatic Heart Disease



## TORULA INFECTION \*

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The existence of torulosis has been known to the medical profession for twenty years or more, yet the disease remains a distinct rarity. Some authors have expressed the opinion that this rarity is more apparent than real and that many cases are passing unrecognized. Nevertheless, there has been no great acceleration in the appearance of case reports which at present number about 50. Of these a few are of dubious authenticity. Although the cultural and morphological characteristics of the organism are fairly well established, animal inoculation experiments have been few, quite variable in result, and frequently incomplete. Because of these considerations certain phases of the literature are reviewed and another case is reported with observations on the bacteriology of the organism isolated and on the pathology of the disease produced in animals experimentally.

Stoddard and Cutler<sup>1</sup> separated torulosis from other yeast-like infections partly on histological grounds. The specific characteristics emphasized consisted of a solution of the tissues about the organism with little or no inflammatory reaction. The organisms were embedded in a gelatinous matrix. The reaction was chiefly monocytic. In the brain these changes resulted in the formation of pseudocysts. When the lesions were massive, caseation occurred. It is to be noted that in the latter event the lesions could hardly be regarded as specific. Subsequent experience in general confirms Stoddard and Cutler's description in all definitely proved cases. The brain lesions are remarkably uniform in character, but most authors describe granulomatous lesions in the meninges and other structures, giant cells being abundant. Necrosis with an exudation of polymorphonuclear leukocytes in the meninges has been reported,<sup>2</sup> and also caseation in the tracheobronchial lymph

\* Received for publication June 7, 1937.



nodes,<sup>3</sup> but in cases without bacteriological control. In local exposed lesions a certain amount of suppuration seems to have been the rule.<sup>4-6</sup>

Torulosis was described originally and many times subsequently as a disease essentially of the central nervous system. However, the literature reveals four general classes of cases dependent on the distribution of the lesions: (1) encephal meningitis alone; (2) central nervous system lesions as part of a more or less generalized torulosis; (3) visceral torulosis without localization in the brain and meninges; and (4) local infections.

Primary torulosis of the nervous system can be established as a reality only on the basis of a most meticulous postmortem examination. Hall, Hirsch and Mock<sup>7</sup> have furnished such a case. The presence of old lesions in the gasserian ganglion, absence of changes in the choroid plexus, and the absence of organisms or lesions in any other organs offering a portal of entry, as shown by cultures and histological examination, led the authors to the conclusion that the Torula had invaded the meninges directly from the nasopharynx. Five other less probant cases have been reported (Hansmann,<sup>2</sup> Freeman and Weidman,<sup>8, 9</sup> Swift and Bull,<sup>10</sup> and Barlow<sup>11</sup>).

More or less general infections with cerebral localization account for the greatest number of cases.<sup>3, 9, 12-20</sup>

The lungs have frequently been described as the principal or unique extracranial focus.<sup>3, 9, 12-14, 20, 21</sup> Other organs that have been involved are the liver (rarely), spleen, suprarenal glands, kidneys, testes, abdominal, thoracic and axillary lymph nodes, bone marrow and the periosteum.

Least known are visceral lesions without brain involvement. Examples are furnished by Gill,<sup>4</sup> Berghausen,<sup>22</sup> and Sheppe.<sup>23</sup> Particularly interesting is Gill's case, because with a less careful laboratory study it would have been classified as a purely local infection. There were abscesses in the ethmoid sinuses, the antrum and hard palate. The organisms were cultured from the blood. The patient recovered and is known to have remained well for 7 years. Sheppe's patient ran a protracted course with joint pains, sore throat and anemia, finally dying with a gelatinous torulous pneumonia. Berghausen's patient had a chronic ulcer of the tongue and died with an unproved pulmonary torulosis. If

torulosis is of more frequent occurrence than the literature reveals, it is in this group of cases and in the following group that failures in diagnosis were made.

There are few reports of local infection. The fact that Gill discovered 3 of these indicates that inadequate bacteriological studies may, in part at least, account for the apparent rarity of this type of case. In the 3 cases of Gill<sup>4</sup> and in the cases of Jones<sup>5</sup> the lesions were located in the nasopharynx or mouth, and had the characteristics of chronic abscesses and ulcers. McGehee and Michelson<sup>6</sup> observed chronic communicating pelvic and inguinal abscesses in a negress. All of these patients recovered.

A coincidence between Hodgkin's disease and torulosis was noted by Mallory (see Cabot's article<sup>17</sup>) who found the coincidence nearly as great as between Hodgkin's disease and tuberculosis. Fitchett and Weidman,<sup>16</sup> on the contrary, believe they have reported the only typical example of this association. The cases of Freeman and Weidman<sup>8</sup> and Smith and Crawford<sup>24</sup> are involved in some doubt.

In mycotic infections one looks for predisposing factors. Berghausen's<sup>22</sup> patient injured his tongue and in this wound the lesion developed. Previous ill health has been mentioned in the cases of Gill,<sup>4</sup> Freeman,<sup>9</sup> Fitchett and Weidman,<sup>16</sup> Cabot,<sup>17</sup> and Williams.<sup>20</sup> All cases seem to have occurred among members of the less fortunate strata of society.

The diagnosis of torulosis must rest on evidence other than clinical and physical findings. In some of these cases the diagnosis has been based on the histopathology alone.<sup>2, 3, 18, 24-32</sup> No cultures were reported by Johns and Attaway.<sup>33</sup> Isolation and identification of *Torula histolytica* is reported by the majority of workers.<sup>5-8, 11-17, 19-22, 34-41</sup> Sheppe<sup>23</sup> presents an unusually complete study. The same is true of Urbach and Zack.<sup>42</sup> The cases of local torulosis reported by Alvarez<sup>43</sup> and Tabor<sup>44</sup> are not due to *T. histolytica* but to some species of pink *Torula*. Badham's case,<sup>45</sup> and that of Swift and Bull,<sup>10</sup> apparently are not cases of torulosis but rather some type of ascomycosis. One of Ball's cases<sup>46</sup> seems given with regard to acceptance of all *Torula* infections as being due to pathogenic species of genus *Torula* Hansen. Some European papers dealing with *Torula* infections, notably the French, to be a monilial infection. Finally a word of caution should be

concern themselves with an entirely different organism under the designation of genus *Torula* Persoon non Hansen.<sup>47</sup> Strictly speaking the latter is better taxonomy. It is unfortunate that these different usages should have occurred. The escape seems to be to drop the term *Torula* entirely and adopt the generic name suggested by Vuillemin,<sup>48</sup> *i.e.* *Cryptococcus*. In this paper we are concerned only with the counterpart of Hansen's *Torula* or the genus *Cryptococcus* of Vuillemin. To conform to current usage we will retain the name *T. histolytica* in this paper.

Animal inoculations that have been carried out since Stoddard and Cutler's study have contributed few new facts and have even obscured to some degree the specific character of the *Torula* lesion. Although originally declared unsuitable, guinea pigs have frequently been employed.<sup>9, 8, 13, 15, 19, 35, 36</sup> Masee and Rooney<sup>15</sup> obtained nodules in the peritoneum in a third of their animals. Lynch and Rose<sup>35</sup> and Watts<sup>13</sup> got negative results. Where the inoculations were positive the lesions were often hardly typical because of a well defined granulomatous reaction with many giant cells,<sup>8, 19, 36</sup> or again they were limited in extent.<sup>8, 15</sup> McGehee and Michelson<sup>9</sup> obtained frank abscesses. On the other hand, typical lesions have been produced in the brain.<sup>8, 19</sup> Rabbits have given irregular results, negative to intraperitoneal and positive to intravenous injections.<sup>36</sup> Sheppe<sup>23</sup> produced a gelatinous pneumonia by intratracheal injections. Fitchett and Weidman<sup>16</sup> inoculated a dog and Barlow<sup>11</sup> a cat intrathecally. Both animals succumbed. In the latter there was a marked inflammatory reaction and evidence that the *Torulae* were merely maintaining themselves. In neither animal did generalization occur. Fitchett and Weidman<sup>16</sup> and Weidman<sup>34</sup> inoculated monkeys subcutaneously and obtained abscesses at the site of the injection which healed rapidly. Fitchett and Weidman<sup>16</sup> obtained typical lesions in rats inoculated intrathecally. That is to say, there were widespread cyst-like lesions in various organs without inflammatory reaction other than a few endothelial leukocytes. Rappaport and Kaplan,<sup>19</sup> by intraperitoneal injection, produced typical brain cysts and also granulomatous lesions with many giant cells in other organs. In Jones' <sup>5</sup> experiment, rats that were inoculated intraperitoneally were living after 3 months. *Torulae* were recovered from nodules in the kidneys. Mice, which are the most susceptible laboratory

animals, have been employed only twice by the various investigators. In one instance results were negative, possibly because the material had been heated.<sup>13</sup> In the other, the lesions showed brain cysts and in all organs the typically feeble inflammatory reaction.<sup>16</sup> From a review of animal experimentation one is not impressed with the specificity of the lesions of torulosis except in the instances where cysts occurred in the brain.<sup>8, 16, 19</sup> Moreover, no satisfactory comparison of results can be made because of the variety of animals used, animals having presumably varying degrees of susceptibility. From this it follows that no information is available concerning the pathogenicity of different strains of *Torulae*.

### REPORT OF A CASE

*Clinical History:* A male negro, aged 25 years, entered the hospital Nov. 27, 1935, complaining of weakness, violent headache and double vision. These symptoms had begun with headache sometime in October, 1935.

Examination revealed some mental clouding, moderate emaciation, great muscular weakness and fever. There was an external strabismus and diminution of vision. The optic discs, particularly the left, were red and somewhat swollen. The tendon and cutaneous reflexes were normal with the exception of the patellars which were absent. Physical findings were otherwise negative.

*Laboratory Findings:* Leukocytes 10,500 falling to 6250. Schilling count 0-1-0-1-7-70-10-11. The cerebrospinal fluid showed increased pressure, sugar negative, globulin + + +, cell count 135. Numerous yeast-like cells, some of them budding, were present. The spinal fluid Wassermann was negative. The urine showed a trace of albumin, smear and culture positive for yeast-like organisms. (Details on blood and urine cultures appear elsewhere in this paper.)

During the subsequent 6 weeks delirium developed, followed by deepening coma with slow pulse and all the usual symptoms and signs of meningitis. Evidence of pulmonary consolidation appeared shortly before death, Dec. 15, 1935.

*Postmortem Examination:* The calvarium was removed in the usual manner. The dura was tense and red. When incised no cerebrospinal fluid escaped. There was only moderate flattening of the cerebral convolutions. The vessels were markedly engorged.

The leptomeninges of both brain and cord showed a diffuse thickening and cloudiness. This was most marked over the brain stem. Everywhere, but particularly along the cerebral vessels, were innumerable, minute yellow masses, none of them more than 1 mm. in diameter. In the sulci there was a small amount of yellowish exudate. After fixation of the brain the tubercle-like bodies were no longer visible, only the cloudiness of the meninges

remaining (Fig. 1). A series of frontal sections showed no appreciable dilatation of the ventricles. They were filled, however, with a colorless gelatinous material. About the lateral ventricles were thickly set cavities varying in diameter from 2 mm. down to the limits of visibility. These likewise contained a gelatinous material (Fig. 2). The choroid plexuses were thick, pale pink and somewhat translucent.

In the lower lobe of the right lung was a patchy gray consolidation and a fibrinous pleuritis. The extreme granularity of the cut surface suggested miliary tubercles with confluence in the denser areas.

Scattered yellow and pearly gray miliary nodules were found on the surface of the left suprarenal gland and throughout the parenchyma of both kidneys (Fig. 3). Lymph nodes were examined with particular care but no lesions were discovered here or in other organs.

*Microscopic Findings:* The leptomeninges were heavily infiltrated by large mononuclear cells and by a few lymphocytes. The majority of the former had the characteristics of compound granule cells and a few of them contained yeast-like organisms. Again there were collections of the organisms without cellular reaction. The lateral ventricles contained innumerable yeast cells and a few degenerating leukocytes, probably monocytes. The adjacent cyst-like structures, described grossly, were filled with yeast cells and a few large mononuclear cells having foamy cytoplasm. The borders of these spaces were ragged and showed no evidence whatever of proliferative or exudative changes. The smallest of them were simply expansions of the perivascular spaces and contained yeast cells alone. The meninges of the cord resembled those of the brain. In the white matter of the cord, about the posterior horns, were numerous mucin bodies. In both brain and cord the nerve cells were in varying degrees of degeneration, mostly mild.

The pulmonary consolidation proved to be a banal bronchopneumonia. The exudate was, however, unusually uniform and consisted almost uniquely of polymorphonuclear leukocytes and fibrin. Torulae, but no other organisms, were found after repeated search (Gram's stain). The Torulae were not believed to have caused the pneumonia.

The lesions in the suprarenal gland and in the kidneys consisted chiefly of punched out cavities containing one or more organisms. An inflammatory reaction was entirely absent or was represented by a few endothelial leukocytes (Fig. 4). Again, endothelial leukocytes and lymphocytes were sufficiently numerous to form a follicular lesion. Only one small Langhans' giant cell was found. Numerous renal tubules were dilated by masses of yeast cells (Fig. 5). In the pancreas there were minute areas of liquefaction necrosis containing yeast cells and strands of collagenous material (Fig. 6). Remarkable was the absence of degeneration immediately adjacent to the lesions. No changes related to the *Torula* occurred in the lymph nodes or spleen.

### LABORATORY DIAGNOSIS

A centrifuged specimen of spinal fluid was examined in a hanging drop. A number of round bodies could be seen. Morphologically these were not unlike red blood cells except that an occasional one was budding. Cultures were made in 1 per cent dextrose veal infusion medium (Difco). After 48 hours incubation at 37° C. a surface growth appeared which on microscopic examination was seen to consist of budding yeast-like cells. Subcultured on Sabouraud's agar a growth was obtained in about 72 hours, which after about 14 days developed a light orange color. Two catheterized urine specimens were examined during the course of the disease. The sediment from the centrifuged specimens was seeded directly on Sabouraud's agar. In both instances growth identical with that obtained from the spinal fluid made its appearance. On two occasions similar organisms were recovered from the blood stream. Ten cc. of blood were inoculated into 50 cc. of brain infusion dextrose broth medium containing some calcium carbonate and brain particles in suspension. In 1 case the growth appeared after 10 days incubation and in another sample the blood culture was positive on the 15th day.

### MORPHOLOGY

Young cells varied in size from about 6 to 12  $\mu$ . In a hanging drop the cytoplasm appeared as a thin gray substance containing small granules. With age there appeared in the culture: (1) an

increase in the number of coarse granules in the cytoplasm; (2) formation of a hyaline cell content by the fusion of the granules; and (3) capsules or shells of varying sizes. These changes among others have recently been elaborated into a life cycle by Todd and Herrmann.<sup>40</sup> Based on the production of tube-like protuberances and fusion of cell contents, these authors believe that there exists a perfect stage in the life cycle of the *Torula* of yeast meningitis. In our preparations tube-like projections were frequently seen. At no time did we observe any fusion of cells. Empty shells were frequent. Cultures on gypsum blocks, sliced carrot, and Gorodkova's agar failed to yield ascospores. No mycelium was ever seen, even in cultures 1 year old. These facts have led us to conclude that the organism in question was *Torula* Hansen non Turpin, Persoon.

### CULTURAL CHARACTERISTICS

The *Torulae* that were isolated grew well on meat extract dextrose agar, Sabouraud's agar and less well on sugar-free agar. Growth was more rapid at 37° C. than at room temperature. Pigmentation could be seen after 7 to 14 days. Various tints appeared: yellow, orange, burnt orange, chocolate, and white colors were seen at some time or other. At no time was the color pink or red. One culture growing on Sabouraud's agar, after a period of 4 months, developed a chocolate brown color. About this time secondary colonies began to make their appearance on the surface of the brown growth. The secondaries were white at first but later turned light orange. Microscopically the cells from the two areas were not remarkably different. This behavior is no doubt an evidence of dissociation and indicates that pigment changes might also be a phase of this process. Up to date no slimy colonies have been encountered. Broth cultures never yielded rings or pellicles, but growth occurred at the bottom in the form of a more or less granular sediment.

### BIOCHEMICAL BEHAVIOR

After 4 days incubation at 37° C. acid but no gas appeared in the tubes containing dextrose and sucrose beef extract broth. In

maltose broth the organism developed at best only a very feeble acidity. No acid or gas was noted in broths containing levulose, lactose, galactose, arabinose, melezitose, xylose, raffinose, dextrin, mannitol, sorbitol, dulcitol, and salacin. Tests for acetyl-methylcarbinol were negative and the organism failed to yield a positive methyl red test. No hydrogen sulphide was formed. This *Torula* failed to hemolyze human blood cells.

### THERMAL DEATH POINT

The authors have found in the literature no record of any thermal death point determinations. Using a 48 hour dextrose agar culture of *Torula* suspended in 10 cc. of peptone broth, pH 7.2, it was found that the organisms were killed by heating in a water bath for 42 minutes at 50° C. At 60° C. the cells were killed in less than 5 minutes. The resistance of the *Torula* is therefore not unlike that of many of the non-sporeforming bacteria.

### ANIMAL INOCULATION

As a preliminary experiment 3 mice were used. Each animal was given an intraperitoneal injection of 0.5 cc. of a 24 hour suspension of *Torula* grown on Sabouraud's agar. The turbidity of the injected suspension stood at No. 10 on the scale of the McFarland nephelometer. One of the animals died after 5 days, the 2nd was killed at this time, and the 3rd mouse lived 8 days. In none of these animals was there any gross pathology. Cultures from the peritoneum, brain, and heart blood yielded *Torulæ* in the two instances where such cultures were made.

The experiment was repeated using 9 mice divided into three groups. Group I received intraperitoneally 0.5 cc. of a 24 hour *Torula* suspension equal in turbidity to No. 1 on the McFarland scale. This was the lightest suspension. Group II received 0.5 cc. of a similar suspension equal in turbidity to McFarland No. 5. This was an intermediate dose. Group III received an equal amount of a suspension corresponding to a turbidity of McFarland No. 10. This was the heaviest dose. The following table sets forth the survivals in this series of animals.



Group I	Survived days
Mouse No. 4 .....	2
Mouse No. 5 .....	17
Mouse No. 6 .....	22
Group II	
Mouse No. 7 .....	9 *
Mouse No. 8 .....	2
Mouse No. 9 .....	40
Group III	
Mouse No. 10 .....	1
Mouse No. 11 .....	2
Mouse No. 12 .....	18

Two guinea pigs were each inoculated intraperitoneally with 1 cc. of a 24 hour growth of *Torula* equal in turbidity to McFarland No. 10. One pig died after 30 days and the other, while still apparently normal, was killed and both were autopsied.

Of the 12 mice inoculated, 10 were completely studied post-mortem. Because neither the dose of organisms injected nor the survival period seemed to have any significant influence on the results, all are considered together.

Only 2 animals failed to show the typical lesions of torulosis. In 1 of these, which had survived 9 days, there were no noteworthy pathological changes. The other survived only 1 day and showed an "acute splenic tumor" due to intense congestion and to crowding of the sinusoids with polymorphonuclear leukocytes.

Positive inoculations resulted in lesions which were similar in all of the mice. Moreover these lesions were similar in most respects to those observed in human cases.

Gross changes were neither conspicuous nor characteristic. On the peritoneal surfaces there was a thin mucoid exudate and there was a gray mottling of the lungs.

Microscopically *Torulae* were found in nearly every organ and even in the skeletal muscle. The predilection of the *Torulae* for the nervous system was apparent because involvement of the brain and the meninges was constant. The brain lesions consisted

\* Mouse No. 7 got its head caught in the cage and was partially strangled, but when released apparently recovered and lived 2 days before death intervened. It had been accidentally inoculated subcutaneously.

of cyst-like cavities of variable size which contained numerous *Torulae*. An inflammatory reaction was utterly absent (Fig. 7). The meninges contained numerous *Torulae* together with only a few mononuclear leukocytes. (The brain lesions in mouse No. 9 fell short of this description in being rare and extremely minute and containing some epithelioid cells.)

Microincineration of the alcohol-fixed brain revealed nothing in the cyst-like cavities except *Torulae* which appeared as bright rings in the dark field.

In the lungs *Torulae* were usually extremely numerous, filling most of the alveoli and occurring in the stroma. Present also were variable numbers of macrophages with abundant foamy cytoplasm and small dense nuclei (Fig. 8). Hyperemia was always moderate and edema was slight or absent.

The lesions in the liver were definitely granulomatous. There were minute collections of monocytes and epithelioid cells among which were rare *Torulae*. In the kidney, suprarenal gland, spleen, myocardium and skeletal muscle the *Torulae* lay singly or in small groups in clear spaces in the interstitial tissues with no trace of an inflammatory reaction about them. The omentum and mesenteries contained enormous numbers of organisms with few or many macrophages. The macrophages were foamy, similar to those observed in the lungs. Of the above tissues and organs, the peritoneum, brain, and lungs were involved in all the animals.

In the guinea pig that was sacrificed no lesions were found. The one that succumbed showed lesions similar to those described in mice. There were large brain cysts but in other organs the lesions were much less extensive.

Concerning the appearances of the *Torulae* in the tissues, they differed in no important respect morphologically from those studied in culture. However, in the peritoneum encapsulated forms were extremely numerous, while in other organs they were rare.

## DISCUSSION

The literature pictures torulosis as a disease that is most often systemic with a cerebral localization. The organism is disseminated by the blood stream from a focus that is commonly found in the respiratory tract. In this form the disease is well known.

On the contrary the non-nervous forms are rare and are involved in certain obscurities. This is the reverse of the situation that exists in regard to the other fungus diseases in which the local manifestations are more common and have been more completely studied than the general manifestations. One may suspect that the picture of torulosis has been distorted because of the relative facility with which the cerebral form may be recognized in the course of a routine lumbar puncture and the difficulties that attend isolation of the organism from visceral or surface lesions.

The case here reported may be considered typical as regards the distribution and histology of the lesions and the bacteriological characteristics of the organisms. Moreover, it has been demonstrated that this typical *Torula histolytica* constantly produces characteristic lesions in mice. It is urged for this reason that in all *Torula* studies mice be used as the standard test animal. Only in this way can the results of different workers be made comparative.

The histology of the lesions of torulosis being quite unique, the mechanism by which the *Torula* acts on the tissues has intrigued certain authors. Stoddard and Cutler regarded the action to be chiefly lytic. Freeman, on the contrary, believed that the clear spaces in the tissues represented an accumulation of the mucoid material elaborated by the parasite. Our own studies leave us in some doubt, but on morphological grounds it appears that both mechanisms are in operation. The manner in which cystic lesions cut across gray and white matter (mice) and the occurrence of similar lesions in the pancreas (human case) where fluid could hardly collect under pressure, speak for lysis. But in the brain lesions there is clear evidence of pressure and even of infiltration about the lesions by fluid. Furthermore, after micro-incineration the products of liquefaction necrosis might be expected to leave a mineral residue which they do not do.

The degree and character of the inflammatory response that the *Torula* excites seem to vary greatly in different human cases. It may be feeble or absent, proliferative or occasionally exudative, and its character cannot be entirely explained by the age of the lesion. In our experimental animals the inflammatory response seemed to be almost entirely histiocytic and its degree related to the local supply of cells capable of being mobilized as macro-

phages. Few in the brain, such cells were more numerous in the lung and peritoneum. The same general statement, applied to our human material, would be an exaggeration, although not an excessive one.

The association of Hodgkin's disease and torulosis raises again the question of the role of infection in the etiology of the former. Because Hodgkin's-like changes have been produced experimentally with a diphtheroid bacillus by Bunting and Yates<sup>50, 51</sup> and with avian and bovine tubercle bacilli by L'Esperance<sup>52</sup> and Medlar,<sup>53</sup> we made a careful search for evidence of mobilization of megakaryocytes, but without success. Fitchett and Weidman<sup>16</sup> report a similar experience.

Concerning the organism itself, a few words at this point might not be out of place. A survey of the literature reveals that there is no specific agreement in regard to the fermentative capacity of the various strains. Pigmentation is also variable. Because of this, fermentation and pigment production can only be given general consideration in any scheme of classification. These variations may be evidence of dissociation. Sheppe<sup>23</sup> has called attention to the production of black "caps" on his colonies. In our own cultures we have observed active dissociation by way of secondary colonies. Fitchett and Weidman<sup>16</sup> mention the formation of mucoid colonies. Punkari and Henrici<sup>54</sup> have studied pigment changes in *Torula*. The detailed variations recorded by different investigators may therefore be related to separate phases of a cyclogeny, a fact well known to occur among the bacteria. In view of this fact, this question interposes itself: How may we recognize *Torula histolytica* for diagnostic purposes? We suggest that the following points are basic to this end: (1) reproduction by budding; (2) absence of mycelium; (3) absence of ascospores; (4) limited powers of fermentation without gas formation; and (5) demonstration of specific lesions in the mouse. In regard to point 3 it is realized that should the work of Todd and Herrmann<sup>49</sup> receive confirmation this criterion will vanish.

Experimental inoculation in mice rapidly results in lesions that are histologically highly specific and remarkably uniform. Because of this fact we suggest that intraperitoneal inoculation of these animals with spinal fluid or cultures should prove a useful method of quickly establishing an exact etiological diagnosis.

## SUMMARY AND CONCLUSIONS

A review of the literature on torulosis reveals that the purely visceral and local forms of the disease, as opposed to the meningo-encephalitic types, have been incompletely studied.

The frequency and the course of these forms can be ascertained only by more complete laboratory studies along the lines suggested in this paper.

Our experience with a case of generalized torulosis indicates the desirability of employing mice routinely for inoculations. This is based on the great susceptibility of these animals and on the uniform and characteristic picture evoked. From this fact we suggest that intraperitoneal inoculation of mice should prove of value in establishing the etiological diagnosis.

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## DESCRIPTION OF PLATES

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### PLATE 122

- FIG. 1. Lateral surface of the left frontal lobe. Thickening and clouding of the meninges, most marked along the cerebral vessels.
- FIG. 2. Multiple small cysts lateral to the ventricles.
- FIG. 3. Miliary lesions in the kidney.
- FIG. 4. Torulae in a glomerulus. The large forms are surrounded by endothelial leukocytes; the small forms are intracellular.  $\times 450$ .



2



4



1



3

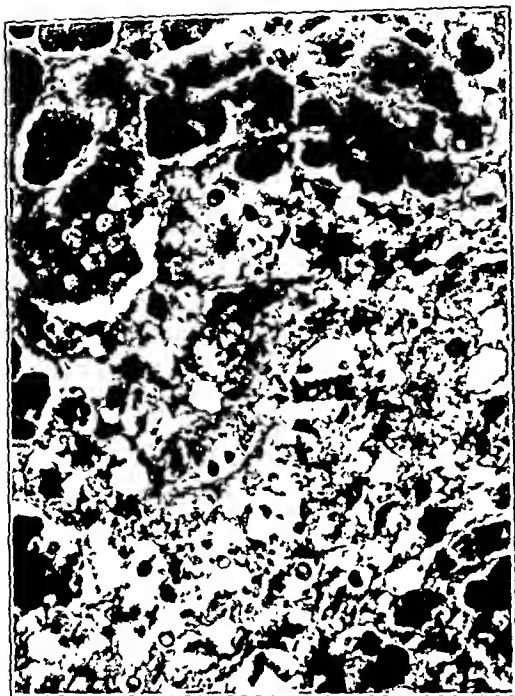
PLATE 123

Fig. 5. Dilated renal tubule containing Torulae.  $\times 450$ .

FIG. 6. Lesion in the pancreas showing the absence of inflammatory reaction.  $\times 450$ .

FIG. 7. Cystic lesion in the brain of a mouse. The cyst is traversed by a capillary containing a Torula.  $\times 450$ .

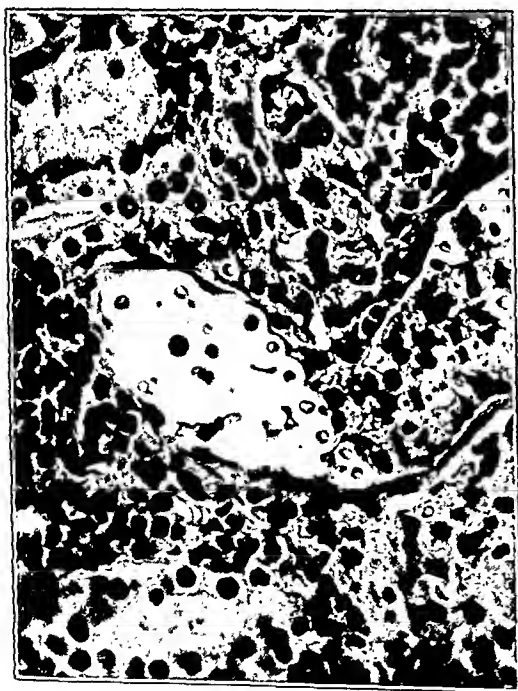
FIG. 8. Torulous pneumonia in the mouse. There are numerous minute intracellular organisms. The exudate consists of macrophages having a foamy cytoplasm.  $\times 450$ .



6



8



5



7

Crone, DeGroat and Wahlin

Torula Infection



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## THE PATHOGENESIS OF DIETARY NEPHRITIS IN THE RAT \*

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Attempts to produce changes in the kidneys of experimental animals which would simulate the pathological alterations observed in diffuse nephritis in man have been made by a considerable number of investigators. A review of the literature on this subject is unnecessary since Horn<sup>1</sup> has recently published an exhaustive general review on experimental nephropathies. Suffice it to say that at present bacterial toxins and parenterally introduced proteins (nephrotoxins, and so on) are considered of greater etiological import in diffuse degenerative changes in the kidney than are dietary factors. Concerning the results obtained from feeding diets containing an excess of protein, Horn comments as follows: "Although tubular lesions may occur, the bulk of the experimental evidence seems to indicate that the degenerative alterations following such a regimen are minimal, and that the only anatomic result is a work-hypertrophy of the kidney consequent to the increased excretion of protein."

In a recent report<sup>2</sup> we have presented the results obtained from a rather extended series of experiments with rats on different dietary combinations. In Table I is presented a brief summary of some of our observations, especial reference being made to the percentage of protein in the various diets and to the number of rats in which the nephritis was of sufficient severity to be the primary cause of death.

\* Received for publication July 6, 1937.

From the table it will be noted that the occurrence of nephritis varied widely with the first 6 diets and that female rats were more refractory than the males to its development. It is apparent that the amount of protein in the diet was not necessarily the determinant as to whether or not nephritis developed (compare Liver Diet XII and Casein Diet I). Another point not shown in the table was that rats with one kidney removed were more liable to develop

TABLE I

*Summary of Previous Dietary Experiments with Special Reference to Percentage of Protein and to Extensive Nephritis*

Type of diet	Protein	Male rats			Female rats		
		Number of animals	Average length of life	Number with extensive nephritis	Number of animals	Average length of life	Number with extensive nephritis
	%		days			days	
Stock II	23.3	19	567	0	32	551	0
Liver I	51.1	26	496	24 (92.3%)	14	523	8 (57%)
Liver XII	22.4	18	395	12 (66.6%)	16	511	5 (31.2%)
Casein I	72.1	6	676	2 (33.3%)	4	722	1 (25%)
Casein II	36.0	6	694	1 (16.6%)	6	631	0
2899, 2899A, 2899 & 2899A modified	25.0 to 27.5	28	563	0	26	563	0
Liver Diets XIV, XV & XX	51.1	..	..	..	18	386	17 (94.4%)

extensive nephritis more rapidly than intact rats. The difference between the male and female rats in their tendency to develop nephritis was thought possibly to be due to a smaller consumption of the diet by the females. Desiccated thyroid was added to Liver Diet I in different amounts with the idea that the metabolism would be increased and that the female rats would thus be induced to consume larger amounts of food. Female rats with one kidney removed were placed on such diets and the results obtained, as shown in Diets XIV, XV and XX in Table I, were comparable with those of the male rats on Liver Diet I. These experiments indicated that the thyroid of itself had some influence on the production of the nephritis since the food consumption was not enough

greater to account for the effects produced. Also, the amount of desiccated thyroid in the diet had a direct bearing on the length of time it took to develop a fatal nephritis. Three rats on a diet containing 0.4 per cent thyroid all developed extensive nephritis with an average length of life of 148 days, and 9 rats on a diet containing 0.1 per cent of thyroid all developed extensive nephritis with an average length of life of 539 days.

During the course of our previous experiments there was an unsuccessful attempt to determine the pathogenesis of the nephritis. Individual variations in the rats and the slowness of development of the nephritis with the diet used defeated the purpose of the experiment. Since Liver Diet XIV was found to induce extensive renal damage in a relatively short time in nephrectomized female rats, it was decided to utilize this diet in a study of the progressive phases of renal damage. The results of these experiments are herein presented.

### MATERIALS

Twenty-four young female rats from which the right kidney had been removed were fed Liver Diet XIV which had the following composition:

Beef liver (dried)	75.0 parts
Lard	15.0 "
Yeast (dried, Harris)	5.0 "
Cod liver oil (Squibb)	3.0 "
Salt mixture (Osborne & Mendel)	1.0 "
Calcium carbonate	0.6 "
Thyroid (desiccated, Armour)	0.4 "

The beef liver was carefully freed from its connective tissue and vessels, cut into small pieces, dried at a moderate temperature, passed through a chopper, dried again at a moderate temperature and then pulverized. Greens were added to the diet twice weekly.

As controls, 12 young female rats, having had a right nephrectomy, were fed Stock Diet II which had the following composition:

Wheat	55.0 parts
Klim (dried milk)	25.0 "
Beef muscle (dried)	12.0 "
Yeast (dried, Harris)	5.0 "
Sodium chloride	2.0 "
Calcium carbonate	1.0 "



The lean beef muscle was carefully freed from fat and connective tissue, passed through a chopper, dried at a moderate temperature and then pulverized. Greens were added to the diet twice weekly.

Stock Diet II was selected as the control diet since previous studies had shown that rats did not develop nephritis when maintained on it.

After being placed on the experimental diets the rats were killed at intervals in groups of three to determine the successive steps that led to the extensive renal changes. Throughout the experiment the animals were kept in separate cages in a room where temperature and air conditions were automatically controlled. A clean piece of blotting paper was placed in the pan of each cage daily except Sunday. Each rat had free access to food and water. Monthly examinations of urine were made to determine albumin values and the presence of casts. To obtain the urine the rats were placed in metabolism cages overnight without food. At the time of killing blood was obtained from each rat for chemical determinations.

Since none of the rats died of nephritis during the limited time set for the study, the tissues from rats on Liver Diet XIV in a previous experiment have been utilized to represent the terminal stages of the pathological process.

### TECHNIQUE

Albumin determinations on urine were made with the sulphosalicylic acid method. Chemical determinations on the blood were made by conventional methods.

The kidneys removed at nephrectomy and at autopsy were cut in two lengthwise, one part being preserved in Zenker's solution and the other in 10 per cent formalin. Paraffin sections were stained with hematoxylin and eosin, phloxine-methylene blue, or Mallory's aniline blue collagen stain without a counterstain. Frozen sections of formalin-fixed tissue were stained with Sudan III to demonstrate fat globules.

### RESULTS

Table II gives a compilation of age, weight, kidney weights and urinary findings for the experimental animals.

TABLE II  
Comparative Data on Nephrectomized Female Rats Fed Stock Diet II and Liver Diet XIV  
STOCK DIET II

[illegible]

## LIVER DIET XIV

1432	46	115	0.50	0.51	21	140	1.52	1.24	160	50	400	584	900	1500	59%	33%	62%	90%	100%
1433	46	130	0.52	0.51	21	95	0.98												
1434	48	125	0.50		21	145	1.22												
1435	46	120	0.49		42	175	1.60			50									
1436	46	130	0.60	0.53	42	175	1.63	1.53	40	100									
1437	48	115	0.49		42	155	1.36		160	200									
1442	36	75	0.31		64	135	2.20		1400	400									
1443	36	85	0.37	0.34	64	175	1.90	2.00	500	400									
1444	36	80	0.33		64	155	1.90		600	800									
1445	37	90	0.38		90	195	2.12		20	100	400								
1446	37	85	0.34	0.36	90	185	2.77	2.40	250	600	400								
1447	37	80	0.36		90	175	2.30		250	750	1000								
1532	31	55	0.25	0.36	106	205	2.01	2.15	75	250	300	500							
1533	32	80	0.42		106	210	2.09		40	250	300	500							
1534	32	75	0.41		106	210	2.09		75	250	300	500							
1547	30	65	0.43		133	205	2.25		75	500	750								
1548	30	70	0.43	0.42	133	210	1.83	2.00	10	150	100	500							
1549	30	70	0.41		133	195	1.92		150	500	1000								
1554	31	75	0.43		155	235	3.54		100	200	500	1000							
1555	41	75	0.37	0.40	155	215	2.72	3.13	40	50	200	500							
1559	37	95	0.38		181	185	2.00		1000	2000	1500	50							
1560	29	45	0.27	0.34	181	180	1.91	2.77	30	120	250	375							
1561	29	60	0.36		181	230	4.40		30	200	500	750							
Range	20-48	45-130	0.25-0.60			95-235	0.98-4.40		10-1400	50-2000	50-1500	584-1000	900-1500	1500-1500	59%	33%	62%	90%	100%
Average	37	87	0.406			182	2.11		231	394	482	584	900	940	59%	33%	62%	90%	100%

The data on the two groups of rats are quite similar up to the kidney weights at autopsy and the urinary findings during the course of the experiment. It will be noted that in the Stock Diet II group the average kidney weight at autopsy was twice that of the

TABLE III  
*Chemical Determinations on Bloods of Rats Used in the Study  
of the Pathogenesis of Nephritis*  
Stock Diet II

Rat No.	Days	NPN	Total protein	Albumin	Globulin	Albumin Globulin	Cholesterol
		mg./100 cc.	gm./100 cc.	gm./100 cc.	gm./100 cc.		mg./100 cc.
1438	33	37.5	5.21	3.06	2.15	1.40	68.3
1439	33	45.2	5.54	3.09	2.45	1.26	83.7
1440	33	44.1	5.62	3.14	2.48	1.27	..
1441	62	35.7	5.94	3.57	2.37	1.51	72.5
1448	58	33.9	5.47	3.61	1.86	1.94	..
1449	58	..	6.48	3.68	2.80	1.32	..
1551	90	35.5	5.83	3.00	2.83	1.06	60.7
1550	90	34.5	5.72	3.22	2.50	1.30	51.5
1552	90	No blood					
1556	148	39.5	5.65	3.24	2.41	1.34	76.9
1557	148	34.6	5.50	3.16	2.34	1.35	70.6
1558	148	35.2	5.67	3.39	2.28	1.49	83.7

LIVER DIET XIV

1432	21	30.8	5.07	2.38	2.69	0.89	86.2
1433	21	49.8	5.37	2.76	2.61	1.06	..
1434	21	34.1	5.21	2.92	2.29	1.28	49.2
1435	42	37.6	5.55	3.18	2.37	1.34	88.9
1436	42	37.3	5.51	3.18	2.33	1.30	81.0
1437	42	34.7	4.84	2.69	2.15	1.25	57.4
1442	64	54.0	5.18	2.80	2.38	1.18	..
1443	64	41.7	4.95	2.76	2.19	1.26	67.8
1444	64	47.6	4.97	2.61	2.36	1.11	68.7
1445	90	24.2	5.07	2.42	2.65	0.91	59.2
1446	90	24.6	4.53	2.08	2.45	0.85	58.0
1447	90	32.6	5.31	2.17	3.14	0.70	76.0
1532	106	29.5	5.79	3.08	2.71	1.13	..
1533	106	29.2	4.94	2.52	2.42	1.04	63.5
1534	106	28.1	5.30	2.73	2.57	1.06	70.9
1547	133	38.5	6.01	3.55	2.46	1.44	108.0
1548	133	34.0	6.12	3.53	2.59	1.36	79.1
1549	133	42.3	5.80	2.89	2.91	0.99	108.6
1554	155	31.2	5.01	2.58	2.43	1.06	88.9
1555	155	33.7	4.88	2.61	2.27	1.15	65.8
1560	181	33.7	5.30	2.71	2.59	1.05	86.2
1561	181	36.8	4.97	2.51	2.46	1.02	96.1
1559	181	34.3	5.30	2.78	2.52	1.10	72.0

kidney removed surgically, whereas in the Liver Diet XIV group the average kidney weight at autopsy was five times that at nephrectomy. This represents in large part a true hyperplasia of renal tissue, as there was no evidence of cystic retention of urine either in the gross or on microscopic examination. The liver diet induced a hypertrophy at least twice that of the stock diet. That the hypertrophy was accompanied by degenerative changes in the kidneys from the rats on Liver Diet XIV is shown by the microphotographs which illustrate the article.

The urinary findings in the two groups are sufficiently different so that no comment is necessary. In our previous report<sup>2</sup> we have noted that the presence of casts and of abnormal albumin values signifies an altered kidney function but does not necessarily indicate that irreparable degenerative changes are occurring in the kidney. We have found, however, that casts and increased albumin output in the urine accompany consistently the pathological alterations that take place in the kidneys of rats on a diet of high liver content and that such a condition terminates in renal insufficiency with death therefrom.

In Table III the chemical determinations on blood are given for the two groups of rats. The data show no significant differences between the two groups. The absence of an increasing deviation from normal in the group on Liver Diet XIV in this experiment as the length of time on the diet increased would indicate that the renal impairment had not progressed far enough to cause retention. These findings agree with our previous observations<sup>2</sup> when we found that renal injury had to be very extensive before retention of nitrogenous products consistently occurred. There was urea retention in every one of 85 rats with a 4 plus nephritis (Figs. 24 and 25), whereas only 23 out of 39 rats with a 3 plus nephritis showed retention. In only 2 rats in our present series, Nos. 1560 and 1561, was the pathological damage severe, and even here it appears that the function of the kidneys at the time the animals were killed was sufficient to maintain fairly normal blood values. Neither of these animals presented lesions that would have been classed as a 3 plus or a 4 plus nephritis in our previous report. The process was not far enough advanced to justify such classification.

The kidney may be regarded as an aggregation of functional

units held together by a framework of reticulum and connective tissue in which the blood and lymph vessels are found. A functional unit consists of a tortuous, epithelial-lined tubule with a complex tuft of blood capillaries (glomerulus), capable of expansion and contraction, invaginated into the beginning of the tubule. The capillary tuft or glomerulus, being covered externally by epithelium, is the region where the blood comes in most intimate contact with the tubular epithelium and where at least the large portion of urinary filtration takes place. From its architecture it seems likely that the separation of substances from the circulating blood is brought about by the glomerular filtration plant and that the tubular epithelium serves mainly to work over, absorb, concentrate and excrete the glomerular filtrate. Considered in this light the functional unit should be considered as a whole since it is improbable that one portion can be severely damaged without the remainder being more or less involved. For simplicity of presentation we choose, however, to divide arbitrarily the functional unit into two parts and to discuss the glomerular and the tubular changes separately.

No abnormal glomeruli were observed in any of the kidneys removed surgically prior to the placing of the rats on the experimental diets. The glomeruli of the kidneys from the rats fed Stock Diet II appeared normal. It is possible that these structures were hypertrophied but of this we could not be certain.

The earliest definite glomerular changes in rats on Liver Diet XIV consisted of small foci in which there were more cells than normal. It seemed as if these foci were composed of cells of the blood or vascular system and that the epithelial cells were, if anything, fewer than normal. The earliest that such lesions were observed was at 60 days. In these early lesions small globules of fat were found on occasion. At this stage the major portion of the capillary bed appeared normal. Later the cells in the focal lesions became large and foamy and fat globules were usually present. As the disease progressed it was possible to find individual glomeruli with focal lesions of different size and appearance (Fig. 7), the latter suggesting a difference in age of the process. This progressive process led eventually to a partial (Fig. 2) or a complete (Fig. 12) sclerosis of the glomerulus.

Increase of the basement membrane (reticulum) of the capillary

tuft, as found in sections stained with aniline blue, was at first focal in character (Fig. 17), corresponding to the small focal lesions, such as seen in Figure 7. Later the reticular increase (Figs. 19, 20, 22 and 23) was found in half or more of the glomerulus. Adhesions between the capillary tuft and the capsular membrane were commonly seen. These adhesions caused a partial to complete obliteration of the capsular space. Increase of the reticulum of the capsular space, with considerable reduplication at times (Figs. 12 and 20), accompanied the increase of reticulum in the glomerulus.

A study of the successive changes in the glomerulus gave a definite impression that the structure became more cellular than normal at certain stages of the process. The increased cellularity appeared to be due to an increase of the epithelial cells covering the capillaries as well as an intravascular accumulation of monocytes. At no time was there found the intense inflammatory reaction (neutrophils and fibrin) which is characteristic of certain acute nephritic lesions in man. No extravasation of blood into the capsular space was evident although on occasion the capillaries were found well filled with blood. At the end stage there were but few cells of any type in the sclerosed glomerulus (Fig. 12). The sclerosing process did not completely obstruct the capillary bed of the glomerulus for even the extensively sclerosed units showed a certain amount of blood within the capillaries.

The afferent and efferent vessels of the glomeruli (Figs. 22 and 23) were not involved in the process taking place within the capillary bed. In other words, the degenerative renal lesions were not dependent on vascular changes, unless those alterations are limited strictly to changes within the glomeruli.

The tubular epithelium and basement membrane appeared normal in all of the kidneys that were removed at operation prior to placing the rats on the experimental diets. No changes of undoubted significance were observed in the tubules of the kidneys from the group of rats fed Stock Diet II.

Significant changes in the epithelium and the basement membrane of the beginning of the tubules (capsules of the glomeruli) were not present in all tubules but they were sufficiently frequent in the group fed Liver Diet XIV, especially in the later stages, to suggest that they were an essential part of the pathological pic-

ture. It seemed as if the primary lesion was an injury to the epithelial cells which in some instances was severe enough to cause necrosis of individual cells. Following this injury there occurred a variable degree of epithelial hyperplasia with more or less conspicuous epithelial crescents (Figs. 10 and 11) being produced at times. These epithelial crescents were suggestive of similar structures present in some cases of human nephritis. Some of the crescents presented the appearance of a syncytium while in others individual cell borders could be distinguished. Thickening of the basement membrane was common in this portion of the tubules. This change seemed to be dependent on injury of the epithelial cells but was not restricted to the areas where epithelial hyperplasia occurred. The thickening of the basement membrane took the form either of broad bands or of splitting (or reduplication) of narrow bands. On occasion (Fig. 20) the split or reduplicated fibers extended in between the epithelial cells of a crescent. These epithelial and basement membrane changes were usually strictly limited to the capsular area of the tubule. On rare occasions similar changes were noted at the beginning of the proximal convoluted tubule (Fig. 19).

Degenerative changes in the proximal convoluted tubules could not be demonstrated. At an early stage the cells appeared to be larger and the granular structure seemed to be more conspicuous than normal. No evidence of necrosis or hyperplasia of the epithelial cells was found. Frequently the lumens of the tubules contained more granular and amorphous material than normal. In the end stages these tubules were often considerably distended with retained glomerular filtrate which in some instances, though not usually, assumed the appearance of casts. On account of the distention, the epithelium often had the appearance of a flattened cuboidal type. On occasion the cells contained brownish pigment which did not give a Prussian blue reaction and was suggestive of urochrome. More than a slight thickening of the basement membrane was rarely found. The increase of the basement membrane when present was possibly a reparative response to the stretching caused by the distention of the tubules.

Changes noted in the loops of Henle and the distal convoluted tubule will be considered together for all of this portion of the functional unit seemed to be simultaneously and equally involved.

At the start there was injury, which sometimes led to necrosis of individual cells (Fig. 18), followed by varying degrees of hyperplasia of the epithelial cells (Figs. 3, 4, 5, 6 and 9). Frequently the hyperplasia was so great that the tubules became packed with epithelial cells (Fig. 21). The appearance of the cells varied considerably. Some were filled with a brownish pigment, suggestive of urochrome; others were replete with large hyaline droplets; and many of the cells contained fat-staining droplets which varied in size and in numbers. Eventually a dissolution of the cells in this region of the tubule occurred with obliteration of the tubular structure.

Changes in the basement membrane (Figs. 18 and 21) accompanied the injury, hyperplasia and dissolution of the tubular epithelium. At first the thickening tended to be focal but later it became general. The increase might appear either as broad bands or as a reduplication of fibers which at times extended in between the hyperplastic epithelium and eventually obliterated the lumen of the tubule.

These tubular changes resulted in areas of fibrosis (Figs. 13 and 14) in which tubular structures had largely disappeared and in which a varying amount of lymphocytic and monocytic infiltration was commonly found (Figs. 12, 13 and 14). These fibrotic areas tended to prevent the outflow of urine and as a result cystic dilatation of the proximal convoluted tubules and capsular spaces of even the remaining normal functional units (Fig. 8) was produced. Such urine retention often caused considerable enlargement of the kidney (Fig. 24), presenting numerous cysts on gross examination.

The only abnormalities noted in the collecting tubules in these kidneys were evidenced in considerable numbers of casts in the late stages of the disease.

All kidneys, even though very extensively involved, have shown some normal functional units. Why some units escape the degenerative changes observed in others is but a matter of conjecture. The pathological process we have discussed above seems to be progressive in nature and it is conceivable that, could the animals survive, all functional units would eventually become involved.



## DISCUSSION

The use of experimental animals provides a method of study of many pathological processes which is superior to material obtained for study from similar lesions in human tissue. Carefully controlled procedures, repetition of experiments and the inclusion of relatively large numbers of animals help to rule out factors which are irrelevant but which from their apparent association with the disease in man may seem to bear a direct and important relation to the pathology encountered. Also it is possible with experimental animals to determine whether the pathological process depends on a single, therefore specific, factor or whether different factors may induce a similar process which thus would suggest non-specificity. There is, however, one thing that must always be borne in mind relative to results obtained from using experimental animals. The biological phenomena of the rat, for example, and of man are not identical. It is quite possible, therefore, that the factors which initiate a pathological process in the rat may differ from those in man and still the pathological picture may be very similar, if not identical, in both instances. In our consideration of the pathogenesis of dietary nephritis in the rat, we are not especially concerned as to the possibility or the probability that the initiating factors for the chronic nephritides in man and in the rat are identical. Since we have been able,<sup>2</sup> under carefully controlled conditions, to bring about consistently a progressive degenerative change in the rat kidney which in the end simulates in many ways the final stage of chronic nephritis in man, we do consider it important to try to evaluate the series of events that take place within the kidney tissue.

It has been our experience that when a group of rats is placed under as similar conditions as is experimentally possible, some of the animals develop nephritis much more slowly than others. These individual variations make it impossible to give a precise chronology to the alterations observed in the kidney tissue. Rather it is possible to discuss only a series of events which seem to dovetail and which in the end lead to a functional and histological destruction of at least portions of functional units.

From the tissues we have studied it appears that the primal renal lesion is a damage to the filter bed of the glomerulus. In just

what way this structure is first affected, or in which stratum, is not clear. Eventually the endothelium, epithelium, and basement membrane are all involved. The primary damage seems to be focal in nature with progressive focal lesions developing, thus presenting lesions of different ages in a single glomerulus. In time the large majority of the glomeruli become sclerosed.

Since abnormal excretion of albumin and casts appears in the urine prior to any demonstrable renal lesions it seems reasonable to assume that the changes found in the tubules result from the presence of substances that have passed through the altered glomerular filter bed. A further substantiation of such a hypothesis is that focal areas of necrosis and hyperplasia of the epithelial cells and of increase of the basement membrane occur in portions of the tubule where there is no intimate contact between intertubular vessels and the tubule structure.

The early injury to the tubular epithelium seldom leads to a necrosis of more than a few individual cells. In the main the injury is sufficient to cause a considerable hyperplasia of the epithelium. This phenomenon is followed later by necrosis and dissolution of the hyperplastic tissue. The whole phenomenon may be regarded as an overwork of the epithelium. The process is selective in type since the proximal convoluted portion of the tubule appears to be little damaged. Richards and Walker<sup>3</sup> have shown that different regions of tubular epithelium perform different functions. It is possible then to attribute the changes noted in the tubular epithelium to a functional selectivity.

The changes that occur in the basement membrane of the tubules depend apparently on the escape through the epithelial covering of substances that cause a considerable increase in the amount of basement membrane material. The process is progressive in nature in the areas of the tubule in which the epithelium is involved, namely the glomerular capsule, the loops of Henle and the distal convoluted tubule. To a considerable degree the amount of distortion of the kidney architecture in the late phases of the disease is related directly to the extent and degree of increase of the basement membrane material. This apparently is the cause of the interstitial fibrosis so evident in extensively damaged kidneys.

In none of the kidneys have we encountered an acute inflammation. In the later stages of the disease lymphocytic infiltration of

the interstitial tissue was a common occurrence and in some instances was pronounced. This we believe is a reaction to damaged tissue and is non-infectious in nature. Including all of our dietary experiments with rats we have not found that spontaneous infections, which have at times been quite common, played a significant rôle in the production of nephritis.

This nephritis, related to certain diets, is a progressive degenerative disease. It appears to begin as a focal damage in the glomerular filter bed and is followed by a selective injury, hyperplasia, overwork and death of the tubular epithelium. Sclerosis of glomeruli and capsules and interstitial fibrosis in the region of the loops of Henle and the distal convoluted tubules represent the reparative end-stage of a burned-out process. The final picture simulates the so-called atherosclerotic nephritis in man with the arteries or arterioles remaining normal.

Smadel<sup>4</sup> has recently reported on the chronic phase of the nephritis produced in rats by "nephro-toxins." It has been the privilege of one of us (E. M. M.) to study sections from some of his specimens. The histological picture presented in Smadel's and in our rats in the chronic phase of the disease is very similar. The difference between the nephritis in the two sets of experiments lies in the fact that we have never encountered the early acute glomerular lesions which Smadel<sup>4</sup> reported. The provocative agent in Smadel's experiments and in our own would appear to be quite dissimilar. It is significant that the end results, in so far as the kidney lesions are concerned, are so similar.

It seems probable that the etiological factors capable of causing progressive degenerative nephritis are multiple and they may well be quite diverse in character. It is also conceivable that the final pathological picture is the same regardless of the nature of the provocative agent. We venture the suggestion that progressive degenerative nephritis hinges on the production of irreparable damage to the filter bed of the glomerulus. This damage may be initiated by toxic products produced during the course of an infection, by so-called nephrotoxins, by unknown abnormal metabolic products or by certain diets (at least experimentally). The sclerosing of glomerular tufts, the obliteration of capsular spaces, the ultimate degeneration of tubular epithelium, the interstitial fibrosis, the inflammatory reaction and the cystic retention probably follow

in the wake of the damaged filter bed. In the progressive degenerative nephritides the chronicity of the disease may well depend on the extent and the severity of damage to the filter apparatus.

### SUMMARY AND CONCLUSIONS

We have previously shown that when rats are fed certain diets of high animal protein content progressive chronic degenerative nephritis can be produced consistently. The pathogenesis of this type of nephritis is here considered. It appears that the initial lesions are focal injuries in the filter beds of the glomeruli and that these are progressive in character. Subsequent to the glomerular damage, injury, hyperplasia and dissolution of the tubular epithelium of the glomerular capsule, loops of Henle and distal convoluted tubule occur. The end-result is a chronic degenerative nephritis in which the principal features are: sclerosis of glomeruli with or without obliteration of the capsular spaces; interstitial fibrosis in the regions of the tubule where the epithelium has been seriously affected; chronic inflammation, which may be considerable; and cystic dilatation of the proximal convoluted tubules, which may or may not be extensive.

The possible relation of experimentally produced dietary nephritis to the chronic nephritides in general is briefly discussed. A suggestion is ventured that chronic degenerative nephritis in general may depend primarily on an irreparable damage to the filter bed of the kidney and that the etiological factors initiating this primal damage may be multiple and diverse in character. We are of the opinion that it is inadvisable to attempt to designate the origin or nature of the etiological agents that induce progressive degenerative changes in the kidney, since information pertaining to the complex phenomenon is so incomplete.

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3. Richards, A. N., and Walker, Arthur M. Urine formation in the amphibian kidney. *Am. J. M. Sc.*, 1935, 190, 727-746.
4. Smadel, Joseph E. Experimental nephritis in rats induced by injection of anti-kidney serum. III. Pathological studies of the acute and chronic disease. *J. Exper. Med.*, 1937, 65, 541-555.

## DESCRIPTION OF PLATES

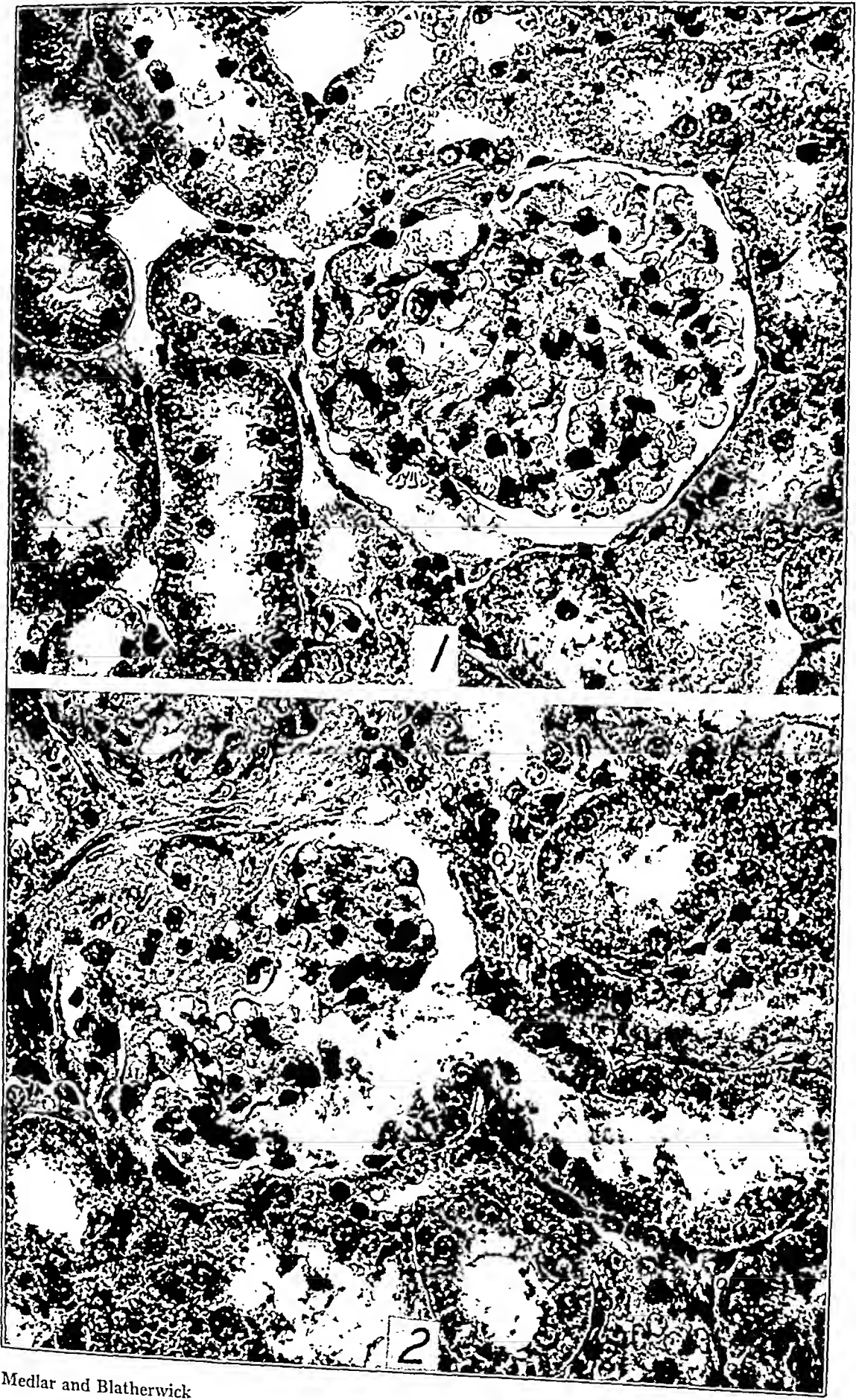
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Figures 1-14 and 24-26 are from sections stained with phloxine-methylene blue. Figures 15-23 are from sections stained with Mallory's aniline blue collagen stain without counterstain.

### PLATE 124

FIG. 1. From the kidney cortex of a rat that had been on Stock Diet II 148 days. The histological picture is essentially normal.  $\times 500$ .

FIG. 2. From the kidney cortex of a rat on Liver Diet XIV for 120 days. Note the focal glomerular lesion with large foamy cells. Note also the granular material within the capsular space and the proximal tubule. The presence of this granular material we interpret as an indication of damage to the glomerular filter bed.  $\times 500$ .

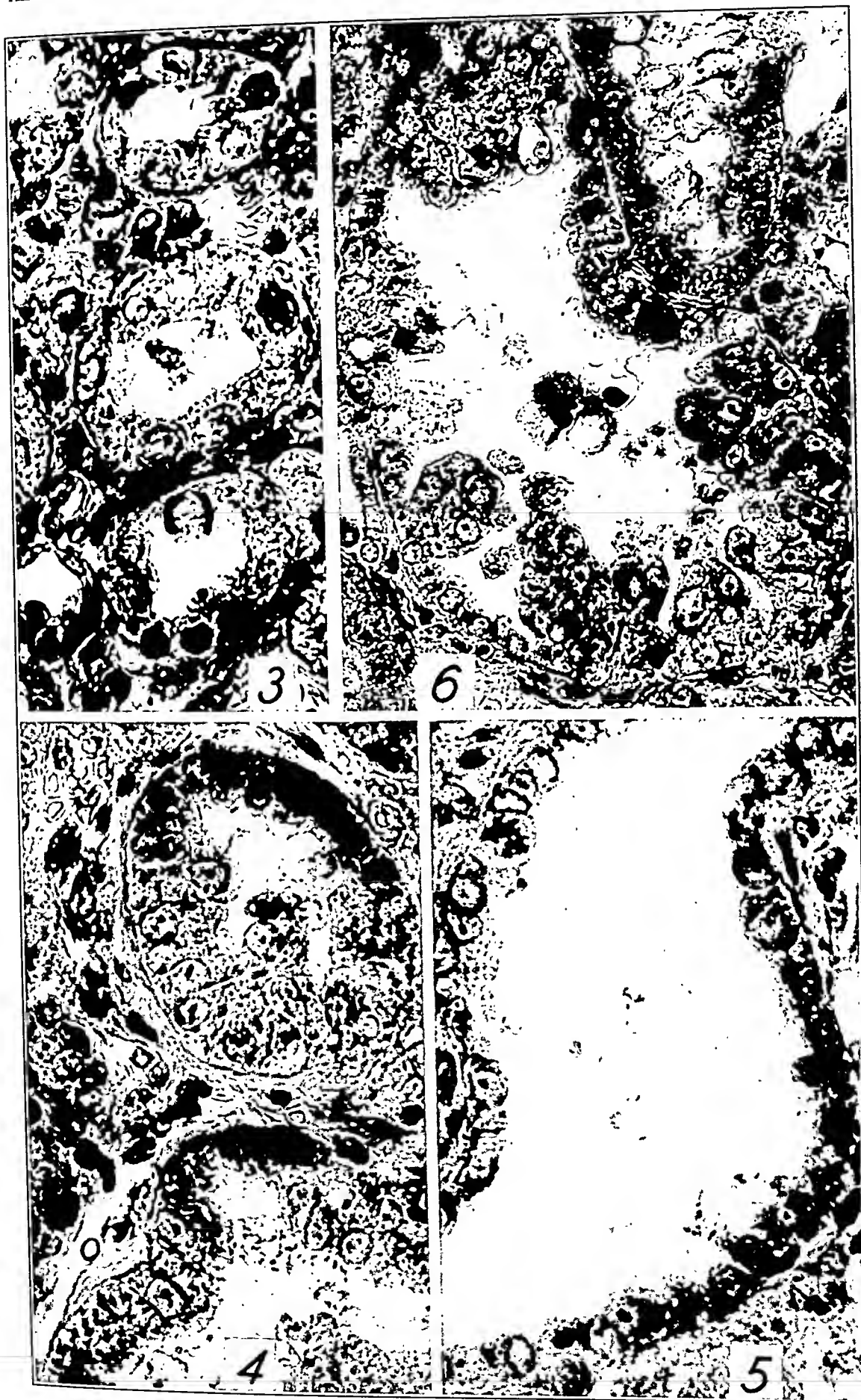


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PLATE 125

- FIG. 3. From the striate zone of the kidney from a rat on Liver Diet XIV for 60 days. Note the mitosis in an epithelial cell of a loop of Henle.  $\times 800$ .
- FIG. 4. From the striate zone of the kidney from a rat on Liver Diet XIV for 120 days. Note the considerable hyperplasia of the epithelium in a loop of Henle. The original epithelial cells form a single layer in the upper quadrant.  $\times 800$ .
- FIG. 5. From a distal convoluted tubule of a rat on Liver Diet XIV for 90 days. Note mitotic figure in upper left hand quadrant.  $\times 800$ .
- FIG. 6. From a distal convoluted tubule of a rat on Liver Diet XIV for 120 days. The hyperplasing epithelium is taking the form of papillomatous projections. A mitosis is present in the group of cells in the upper portion of the tubule.  $\times 500$ .



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PLATE 126

FIG. 7. A glomerulus from a rat on Liver Diet XIV for 90 days. There are three focal lesions; left center, lower center and upper right border. The older lesion is composed of large foamy cells (fat-containing), probably monocytes. Focal lesions of different ages were often observed. Note the early crescent of capsular epithelium to the right.  $\times 1000$ .

FIG. 8. A fairly normal functional unit from a rat on Liver Diet XIV for 10 months. In this particular kidney such units were scarce. The dilatation of the capsular space and proximal convoluted tubule is caused by interstitial fibrosis in the striate zone of the kidney.  $\times 500$ .

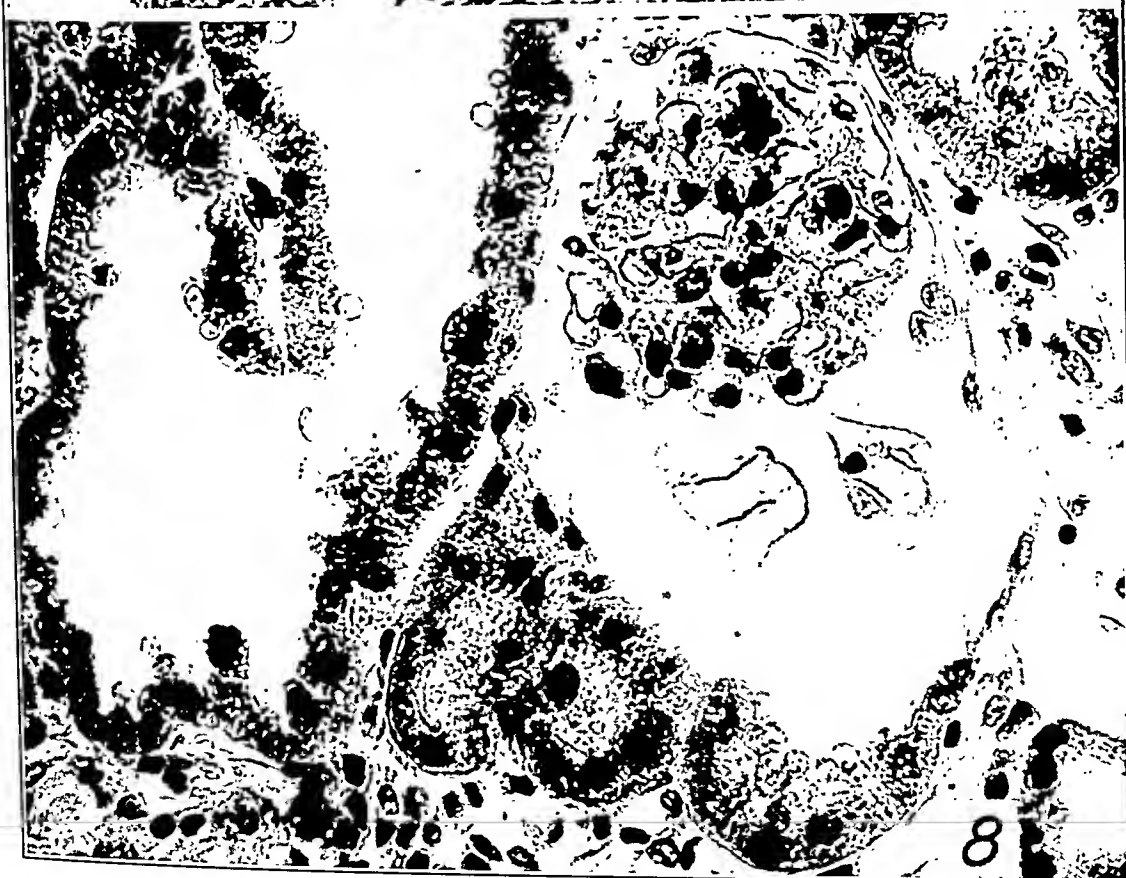


PLATE 127

- FIG. 9. Tangential section of loop of Henle from a rat on Liver Diet XIV for 90 days. Note focal regenerated epithelium and thickened basement membrane along lower portion of tubule. This lesion is not in the proximity of intertubular vessels which are to the extreme left. The rest of the tubular epithelium has a frayed-out appearance. Note also that the tubule is filled with amorphous material which probably is glomerular filtrate.  $\times 500$ .
- FIG. 10. Glomerulus from a rat on Liver Diet XIV for 180 days. Note especially the crescent of capsular epithelium and the thickened basement membrane.  $\times 500$ .
- FIG. 11. Glomerulus from a rat on Liver Diet XIV for 150 days. The lower half of the glomerular tuft is pretty well sclerosed. A typical crescent is present.  $\times 800$ .

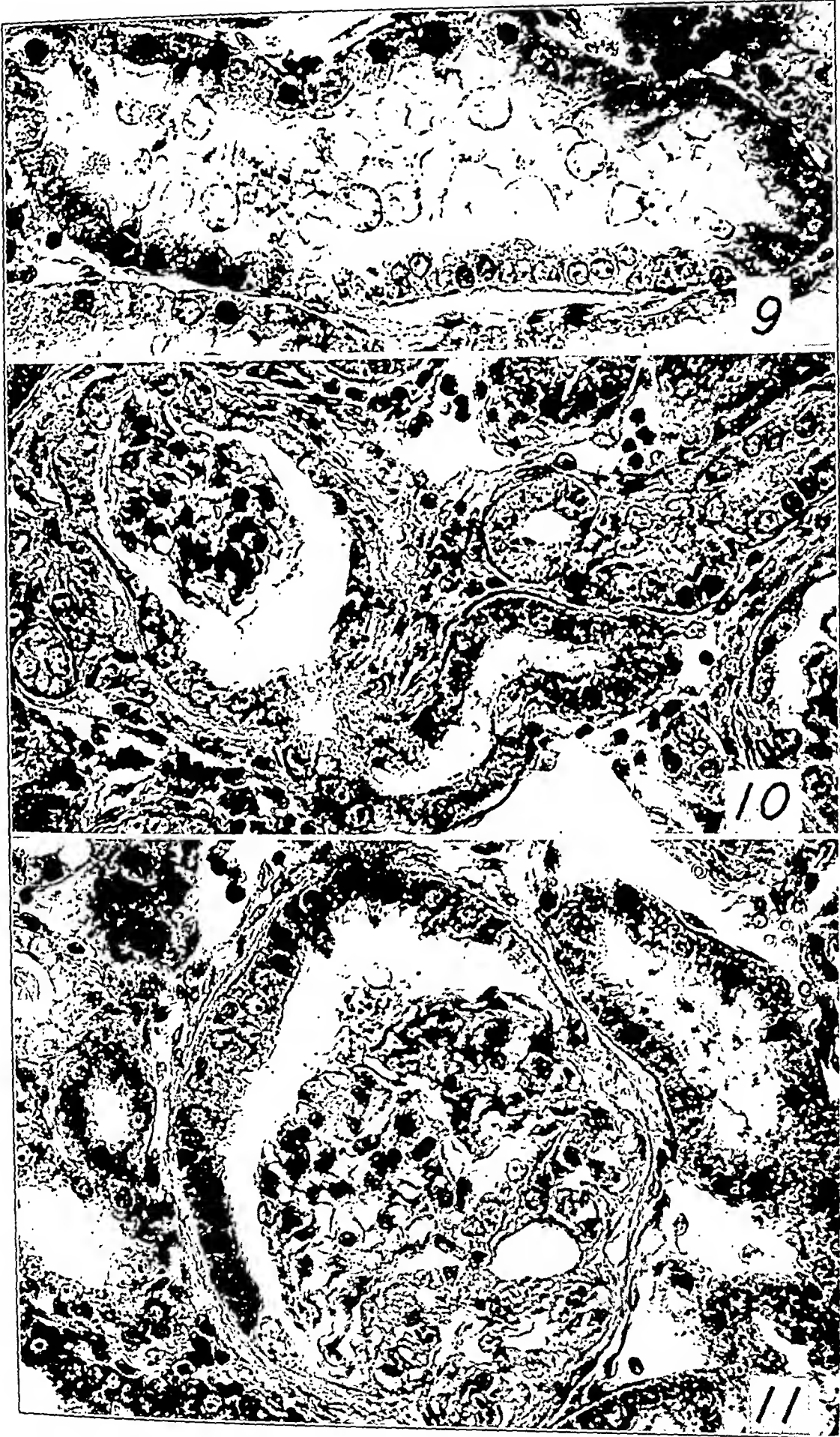


PLATE 128

- FIG. 12. A sclerosed glomerulus with obliteration of the capsular space from a rat on Liver Diet XIV for 208 days (from kidney shown in Fig. 25). Note the lymphocytic infiltration in interstitial tissue adjacent to glomerulus. The infiltration in this illustration is mild when compared to that of other areas of the same kidney. In this particular kidney uninvolved glomeruli were few in number.  $\times 500$ .
- FIG. 13. An area in the striate zone of the same section from which Figure 12 was taken. Note fibrosis, lymphocytic infiltration and lack of tubular structure.  $\times 500$ .
- FIG. 14. Another area in the striate zone from the same section from which Figures 12 and 13 were taken. Here the lesion is evidently an end stage with the fibrosis being the principal feature. The central portion of the picture suggests a fibrosed tubule similar to one shown in Figure 6 with two or three single epithelial cells remaining.  $\times 500$ .

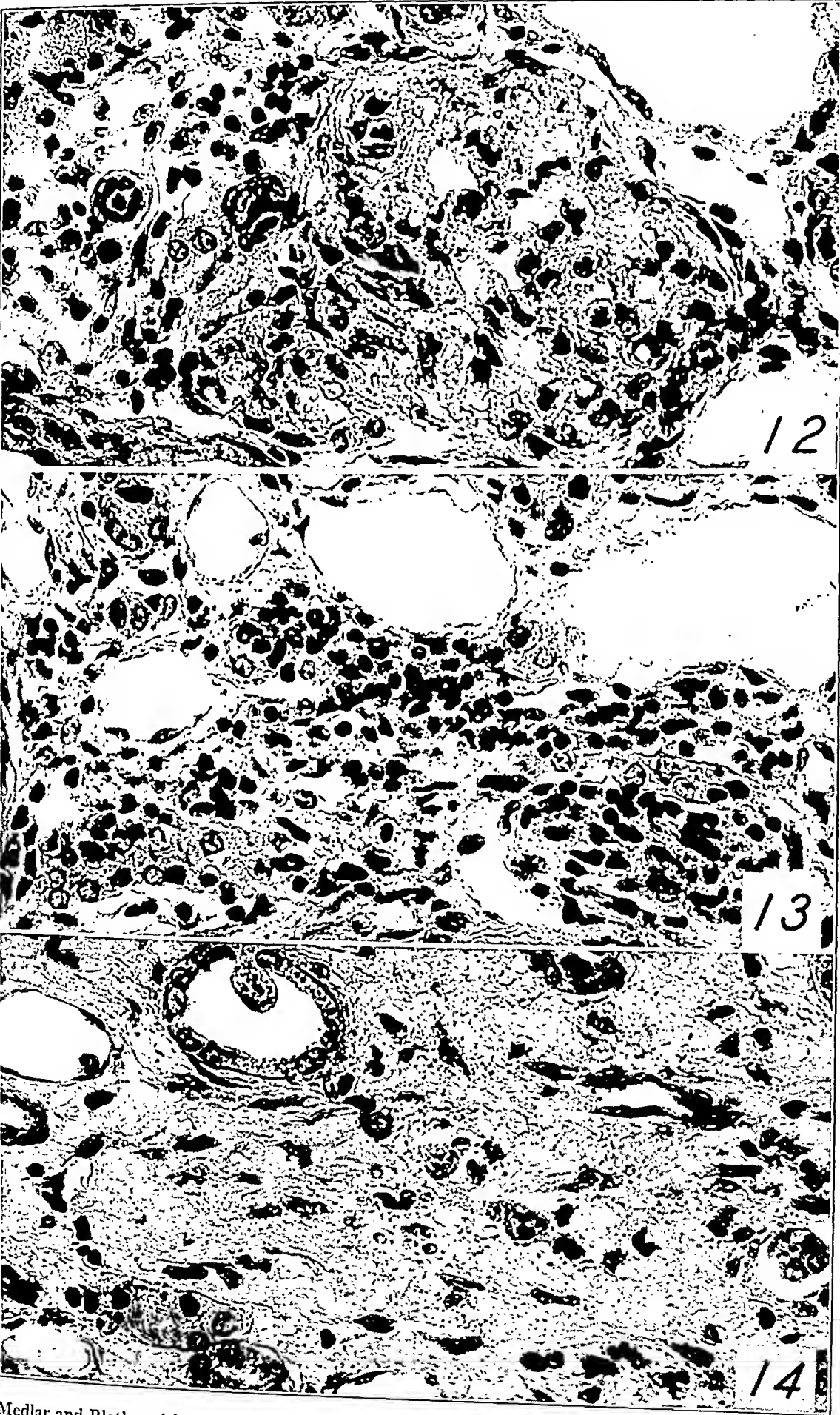
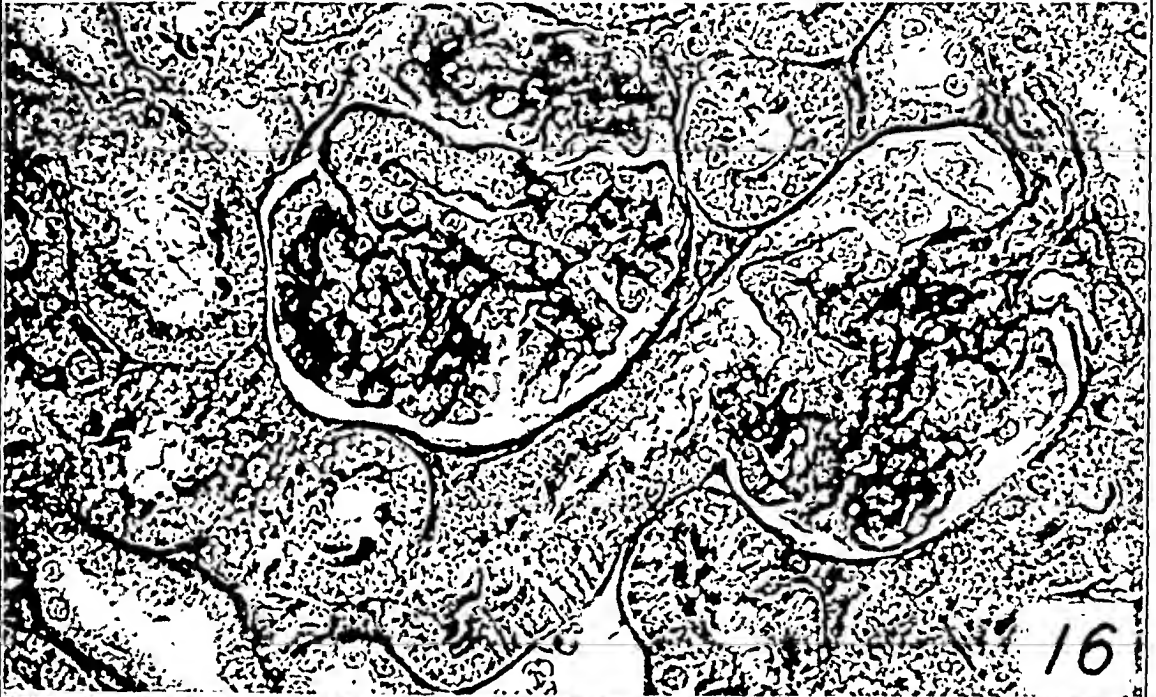


PLATE 129

- FIG. 15. From the striate zone of a kidney from a rat on Stock Diet II for 148 days. This shows the normal basement membrane (reticulum) pattern.  $\times 500$ .
- FIG. 16. From the cortical zone of the same kidney section from which Figure 15 was taken. This shows the normal reticulum pattern for glomeruli and tubules in this region.  $\times 500$ .
- FIG. 17. Glomerulus from a rat on Liver Diet XIV for 60 days. Note the small focal thickening of the reticulum.  $\times 500$ .





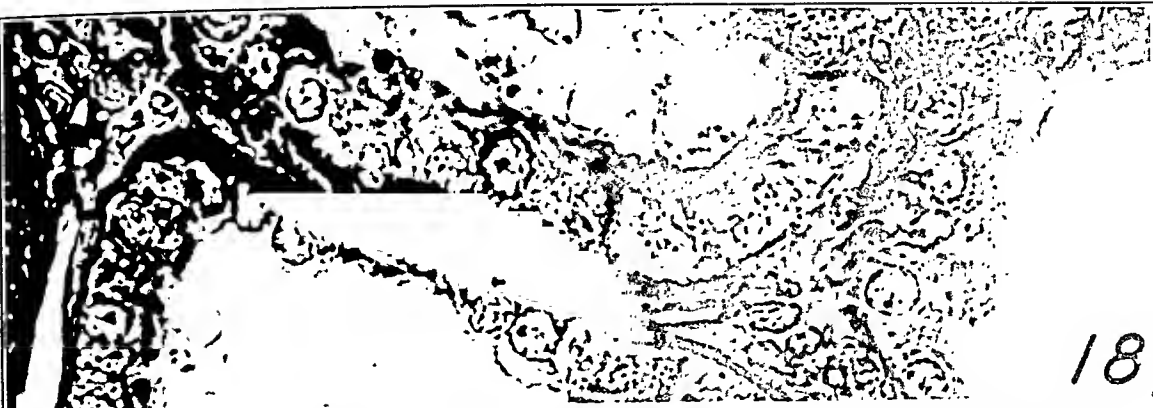


# PLATE 130

FIG. 18. From the same kidney from which Figure 9 was taken. On Liver Diet XIV for 90 days. Note focal thickening of basement membrane and two necrosed cells just below the thickened area. Such lesions are frequently found and seem to depend on material escaping from the tubule. Note also that the intertubular vessels lie to the right and left and are not in juxtaposition with the thickened membrane.  $\times 800$ .

FIG. 19. From the same kidney as Figure 10. On Liver Diet XIV for 180 days. Note focal increase of reticulum in the tuft, the partial obliteration of the capsular space and the thickened membrane of the capsule which involves the beginning of the convoluted tubule. This latter lesion is not commonly seen.  $\times 500$ .

FIG. 20. Glomerulus from a rat on Liver Diet XIV for 180 days. Note especially the reduplicated fibers of the capsular basement membrane which to the left extends between the epithelial cells of a crescent. Although the sclerosis is pronounced the capsular space is only partially obliterated.  $\times 500$ .



### PLATE 131

- FIG. 21. From the striate zone of a kidney from a rat on Liver Diet XIV for 150 days. Tubules are filled with epithelial cells, many of which are disintegrating. The heavily stained cells are full of pigment. Around the larger loops of Henle the basement membrane fibers tend to be reduplicated while around the thin portions of the loop the basement membrane tends to remain in a much broadened band. Compare with the basement membrane pattern shown in Figure 15.  $\times 500$ .
- FIG. 22. A glomerulus from the same section from which Figure 20 was taken. Note focal thickening of reticulum in the tuft and the "adhesion" extending from the tuft to the capsular basement membrane. Note also that the afferent and efferent vessels to the left are normal.  $\times 500$ .
- FIG. 23. A sclerosed glomerulus from the same kidney from which Figure 12 was taken. Note especially that the vessel outside of the glomerulus is normal. In none of the kidneys have there been sclerotic changes in the arteries or arterioles. The pathology is confined primarily to the capillary tufts and the tubules.  $\times 500$ .



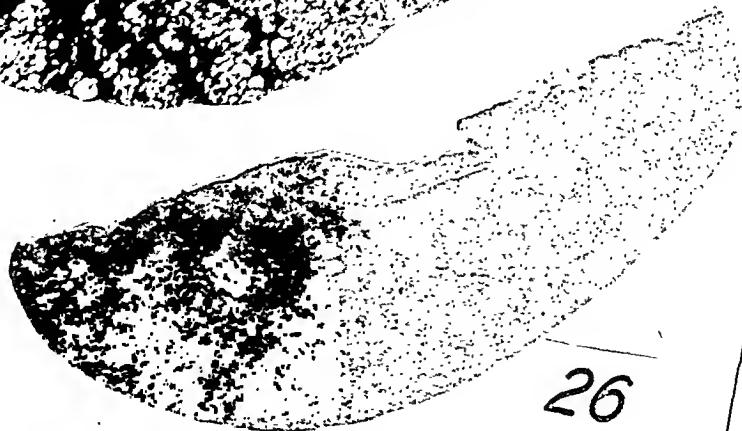
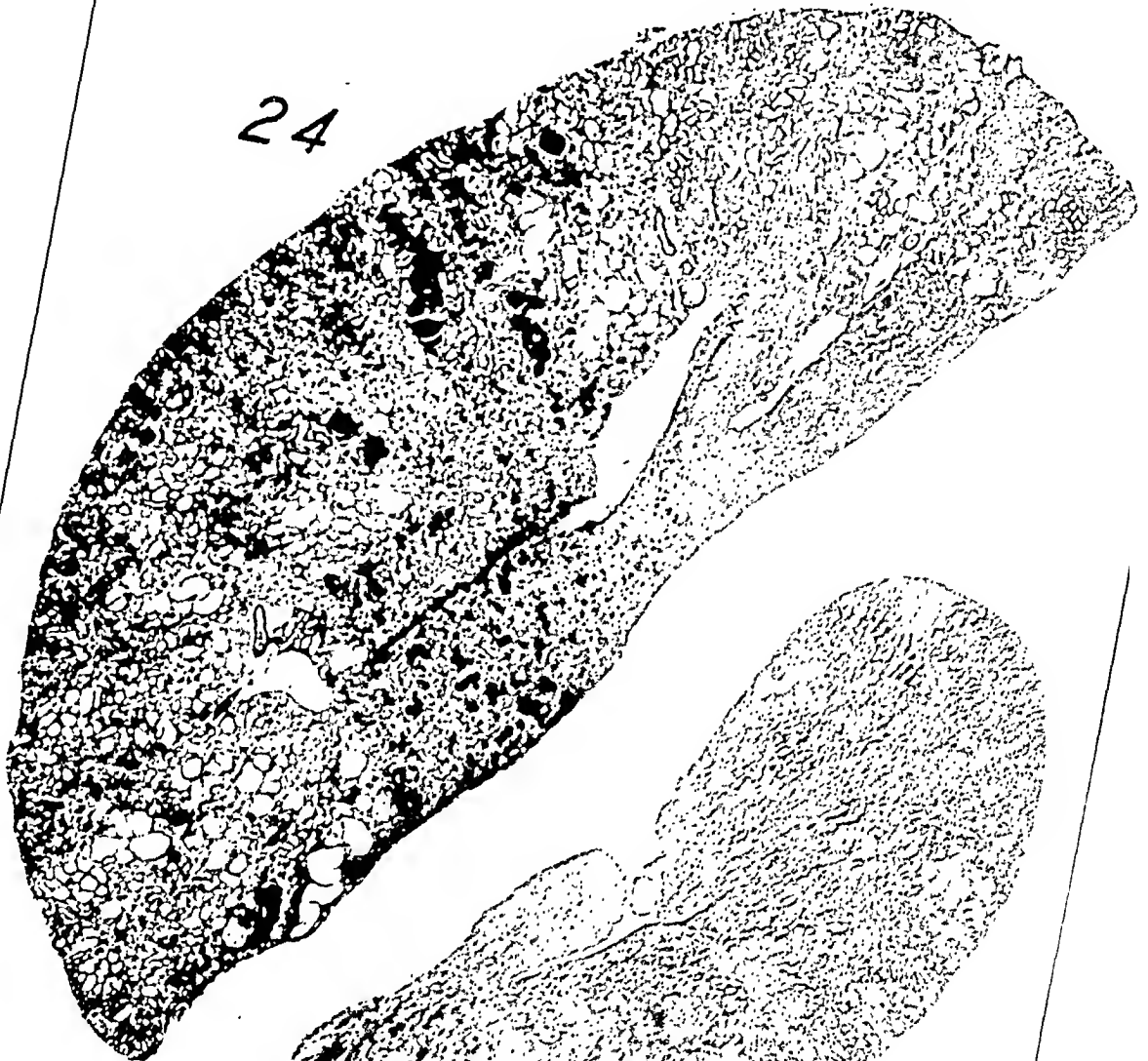
PLATE 132

Microphotographs of comparable section from the left kidneys of 3 rats (from previous studies) to illustrate differences in size and general structure. Each of the rats had a right nephrectomy prior to being fed the experimental diet.

FIG. 24. Rat on Liver Diet XIV for 156 days. Died uremic. Kidney weighed 8.52 gm. Microscopic examination showed: Extensive glomerular involvement and fibrosis; great cystic dilatation of tubules; large numbers of tubular casts (the very dark areas in the illustration). Classed as 4 plus nephritis in previous report.  $\times 8$ .

FIG. 25. Rat on Liver Diet XIV for 208 days. Died uremic. Kidney weighed 5.40 gm. Microscopic examination: Similar to Figure 24 except that there was less cystic dilatation of the tubules and there were very few tubular casts. The difference in weight of the two kidneys is apparently due to a greater retention of urinary secretion in one than the other. Classed as 4 plus nephritis in previous report.  $\times 8$ .

FIG. 26. Rat on Stock Diet II for 658 days. Animal in good condition when killed. Kidney weighed 2.17 gm. Microscopic examination: The organ presented a normal histological structure and was so reported in a previous article.



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## THE INFLUENCE OF ALLERGY ON THE DEVELOPMENT OF EARLY TUBERCULOUS LESIONS \*

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The influence of bacterial allergy in the form of tuberculin hypersensitiveness in accelerating the development of tubercle formation has long been generally recognized, and certain of its effects such as the intensification of inflammatory responses and the production of necrosis have been repeatedly emphasized. That allergy might play an even more fundamental rôle in the pathogenesis of the disease by determining, in part at least, the character of the anatomical lesions of tuberculosis has received little consideration. Yet, as the authors have pointed out in a previous paper,<sup>1</sup> the similarity of the histological reactions in most of the so-called infectious granulomas — syphilis, typhoid, glanders, brucellosis, tularemia and lymphogranuloma inguinale, for example — is so great that most pathologists candidly admit the dangers of confusion in routine diagnostic work. In all diseases of this group focal tissue reactions characterized by a predominance of large mononuclear phagocytes are the rule and in this same group of diseases bacterial allergy — defined as the first stage of the immune response to parenteral antigen, characteristic only of active as contrasted with passive immunity — is most regularly and persistently present and most highly developed.

Such a constant association of a morphological picture with an immunological state inevitably suggests the possibility of a causal relationship, though a more complicated interdependency cannot be excluded. If the relationship is a simple one, our alternatives are either that the presence of granulomatous lesions predispose to the development of a particularly high degree of bacterial allergy, or that bacterial allergy determines a focal mononuclear type of cytological response on the part of the host. That the latter actually occurs was the conclusion drawn by the authors in the work already cited and since confirmed in all essential details by Laporte.<sup>2</sup> Rössle,<sup>3</sup> too, was evidently thinking along closely similar lines when he recently stated that granulomatous reactions can

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occur only in diseases in which allergy develops. For a more complete discussion of the relationship of allergy, not only to cytological responses but to the production of antibodies, the reader is referred to the review recently published by one of the authors.<sup>4</sup>

So far as any theory of the histogenesis of the tubercle may be said to have received general acceptance, the tubercle is usually looked upon as analogous to a foreign body reaction to a parasite which is relatively indestructible and possesses a high lipid content. Yet quantitative considerations of the amount of lipid in a few hundred tubercle bacilli, compared to the amount of tubercular or other lipid required to produce a noticeable foreign body reaction, obviously render such a hypothesis inadequate. Moreover, such an explanation could never even be promulgated to explain the nearly identical reactions to such different parasitic antigens as Gram-negative bacilli, spirochetes and filterable viruses.

The present work has, therefore, been undertaken to test further the applicability to tuberculosis of the hypothesis that allergy is the primary factor in determining the character of the early histological response to the infection in susceptible animals. If such a theory is tenable, it must be demonstrable (1) that the granulomatous lesions are not essential to the development of the delayed type of allergy, and (2) that tuberculin sensitivity develops at least as early as the appearance of the characteristic histological picture in the initial tuberculous lesion. The first of these points was demonstrated by Dienes<sup>5</sup> some years ago in the following manner. Guinea pigs were injected intratesticularly with a large dose of tubercle bacilli. Twenty-four hours later, when control histological examinations showed simple abscess formation with a practically pure polymorphonuclear response and without a trace of the granulomatous reaction considered characteristic of spontaneous or experimental tuberculosis, a simple protein such as egg albumin was injected into the testicle. After a second 24 hour period the testicle, still free from any trace of granulomatous reaction, was excised. The animals, however, proceeded to develop after 3 to 4 days a high grade of delayed hypersensitivity of the tuberculin type to the injected egg albumin. Furthermore, in a later work Dienes and Mallory<sup>1</sup> showed that typical, albeit rather feeble, delayed sensitivity to non-bacterial proteins could be regularly produced in uninfected animals. The present experiments are, therefore, devoted to testing the second of the imposed conditions

by an analysis of the time factors governing the development of hypersensitiveness and the appearance of mononuclear infiltration, fibroblastic proliferation and other factors of the granulomatous tissue response.

## EXPERIMENTAL

### *Methods*

The general plan of the experiments was as follows: Guinea pigs were heavily infected with large doses of tubercle bacilli to ensure rapid development of detectable tuberculin sensitivity. Pairs of animals were later reinfected with small doses of bacilli at suitably chosen times so that there were available for histological study reinfectious lesions produced from 1 to 8 days after the first infection and from 1 to 3 days old. The sites of inoculation and of reinfection were varied in different sets of experiments to control as far as possible the effect of varying tissue substrates, and lesions of the omentum, the skin, the loose tissues of the groin and of the testicle were studied. In a preliminary series of 10 animals the first infection was in the peritoneal cavity; in the 3 subsequent series, comprising in all 29 animals, the first infection was testicular. In the first 3 series of experiments the R<sub>1</sub> low-virulent strain of tubercle bacilli was used in 10 mg. doses for the primary infection, and in 0.1 to 2 gm. doses for the reinfectious lesions. In the final set of 9 animals 5 mg. of a freshly isolated, virulent human strain was employed for infection, and 0.1 mg. of the same strain for reinfection. All animals were skin tested with 0.01 cc. of an unheated synthetic medium tuberculin which gave no reaction in uninfected pigs to determine the first appearance of tuberculin sensitivity. At appropriate intervals the animals were killed, the lesions — primary infections, reinfections and tuberculin reactions — excised, fixed in Zenker's fluid, stained with hematoxylin and eosin or eosin-methylene blue, and with Ziehl-Neelsen's stain. Serial sections were not made but 3 sections at different levels from each block usually sufficed to give satisfactory pictures of the lesions. Since the relative distribution of granulocytes and mononuclear phagocytes characteristically showed a reciprocal relationship in proportion to the distance from the center of the lesion, care was exercised to use for study only sections that could be shown to pass close to the center of the lesion.

*Experiments*

In the preliminary series 20 mg. of the R<sub>1</sub> strain was injected intraperitoneally and at the same time a dose of 2 mg. was given intracutaneously. Pigs were killed on the 2nd, 3rd, 4th, 6th, 7th and 9th days. Each pig had been injected intracutaneously with a small dose varying from 2 to 0.1 mg. R<sub>1</sub> bacilli the day before death. The peritoneal, the original intracutaneous and the 24 hour cutaneous lesions were all examined grossly and histologically. From the gross point of view the peritoneal lesions developed, as was expected, primarily in the omentum, though in a few animals lesions were seen in the mesentery or the tunica as well. Up to and through the 3rd day the omentum, though rolled up into a ball, swollen and hyperemic, could be fairly easily untwisted by gentle traction, but by the 4th day isolated firm lesions became evident and on subsequent days dense fibrous adhesions bound it into a solid tumor-like mass. Microscopically by the 3rd day mononuclear cells predominated and from the 4th day onward fibroblastic proliferation was evident, which rapidly resulted in the development of abundant granulation tissue.

In each of the guinea pigs killed from the 3rd day onward two skin lesions were available for study, one made simultaneously with the intraperitoneal injection, the second 24 hours before the pig was sacrificed. In the 3 day pigs both the 72 and the 24 hours old reactions were similar in character though materially different in extent. The reaction in each consisted essentially of polymorphonuclear infiltration with very few mononuclears and no evidence of fibroblastic proliferation. Reactions from the 4th day on — the 24 hour as well as the older reaction — were entirely different in character. Polymorphonuclear leukocytes were still abundant and tended to be clustered fairly compactly about the bacilli, but the surrounding tissue showed an extensive reaction with many mononuclear phagocytes hovering about the edges of the lesions and clumped about the blood vessels and nerve sheaths.

This preliminary set of animals served to emphasize the significance of the period from the 3rd to the 5th day, and in the subsequent series attention was especially concentrated on this period.

In several of these animals an interesting phenomenon characterized by a fiery red, hemorrhagic reaction of the parietal peri-

toneum developed. A similar local hemorrhagic reaction was noted in some of the animals described below in the group recorded as Series III, which were infected in the loose tissues of the groin.

A brief description of this phenomenon seems justified since we are unaware of any published report of a similar phenomenon in uncomplicated tuberculous infections, though Dienes <sup>6</sup> has reported a similar lesion following the injection of egg white in tuberculous animals previously infected and sensitized by the intraperitoneal route. It was observed in about half of the intraperitoneally infected animals, which were sacrificed on the 5th, 6th and 7th days after infection, and in one instance was observed by laparotomy under local anesthesia to rule out agonal phenomena. The reaction consisted of multiple small hemorrhages, sometimes nearly confluent, in the parietal peritoneum, which ordinarily shows little or no gross change at this stage of intraperitoneal infection in contrast with the omentum which is heavily beset with lesions. It was particularly evident where bits of omentum or of exudate had become adherent to the peritoneum. Microscopically these areas showed in addition to the hemorrhage intense monocyctic infiltration, but no polymorphonuclear accumulation such as invariably is associated with the presence of the organisms themselves (Fig. 12).

In the guinea pigs of Series III a 5 mg. primary dose was given simultaneously in the groin and in the testicle. In the latter no hemorrhagic phenomenon appeared, but in the former 3 days after infection a zone of hemorrhage 30 mm. in diameter developed, which reached its maximum on the 4th day and then slowly disappeared during the next 4 days. A similar reaction has been noted in rabbits infected in the subcutaneous tissues.

Both the peritoneal and the subcutaneous lesions resemble closely both grossly and microscopically the flaring up of infection sites at the moment when allergy develops, and also the similar reactions recently reported from this laboratory following injection of human serum and of turtle egg in guinea pigs.<sup>7</sup>

It seems evident that these reactions are allergic in character and it is suggested that they indicate diffusion for some distance from the lesions through the tissues of tuberculin-active substances. This in turn renders possible the early sensitization of tissues in the immediate neighborhood of a lesion before generalized sensitivity develops.

Such features of the subsequent 3 series of experiments as are susceptible of tabulation are presented in Table I. From this it will be evident that no animal proved tuberculin-sensitive on the 2nd day, and no animal failed to show definite sensitivity on the 4th day. The reactions on the 3rd day appeared negative or equivocal on gross examination. When examined microscopically, using the criterion of significant mononuclear infiltration as evidence of a positive reaction, 3 out of the 7 examined microscopically were considered weakly positive.

The histological preparations of the tuberculin reactions showed the picture that we have previously described<sup>1</sup>; a very slight and presumably non-specific reaction to the injected fluid characterized by a small number of scattered polymorphonuclear leukocytes in the 1st and 2nd day, and most of the 3rd day animals; marked constant mononuclear infiltration in all animals tested from the 4th day onward, with some tendency to the reappearance of polymorphonuclears as the degree of sensitiveness became more intense and necrosis appeared in the tissues. In a recent paper which essentially confirms our observations Laporte<sup>2</sup> describes edema and polymorphonuclear infiltration as the first stage of the histological process in a tuberculin reaction. At a later point in the paper he describes a similar result from injecting tuberculin into normal animals. Our findings are entirely in accord with his but we prefer to regard this reaction as a non-specific response to the trauma of the injection and to the faintly irritating effect of all tuberculin preparations rather than as an integral part of the tuberculin reaction.

In the reinfectious lesions (*i.e.*, intracutaneous or intratesticular injections of living tubercle bacilli in a previously infected pig) the invariable initial response was a flood of polymorphonuclear leukocytes which quickly clustered about the organisms with the formation of small abscesses. When the reinfection was produced 2 days after the primary lesion nothing more was noted, but in some of the 3 day examples, and constantly from the 4th day on, a noticeable alteration in the reaction appeared. There was greater hyperemia, the fibroblasts of the surrounding tissues appeared swollen and their nuclei hyperchromatic; in the early stages an occasional mitotic figure, and in the later stages numerous mitoses were found. Simultaneously, as far as we could judge, large num-

TABLE I

Day	Series I										Series II										Series III										
	Primary infection — 10 mg. R <sub>1</sub> intratesticular Reinfection — 0.1 mg. R <sub>1</sub> intracutaneous										Primary infection — 10 mg. R <sub>1</sub> intratesticular Reinfections — 0.1 mg. intracutaneous										Primary infection — 5 mg. virulent Tb., testicle or groin Reinfections — 0.05 mg. virulent Tb., skin and testicle										
	Primary lesion			Tuberculin			R <sub>1</sub> intracutaneous				Primary lesion			Tuberculin			R <sub>1</sub> intracutaneous				Primary lesion			Tuberculin			Virulent Tb. intracutaneous				
	P	M		Gross	P	M	Gross	P	M		Gross	P	M		Gross	P	M		Gross	P	M		Gross	P	M		Gross	P	M		
2	+++	±		3 x 4	±	±	3 x 4	+	o		+++	±		Trace	±	o	o	++	±	±		+++	o		o	++	±	±	o		
3	+++	+++		5 x 6	±	±	5 x 6	+	o		+++	±		Trace	±	o	o	++	±	±		+++	±		Trace	Trace	+++	+	+	o	
4				3 x 4 5 x 6	±	±	5 x 6 5 x 6	+++	+		+++	+++		Trace Trace Trace	±	±	±	Trace o Trace	+++	±		+++	+++		Trace Trace	+++	+	+	+	+	
5	++	+++									+++	+++		7 x 7	±	+	10 x 8	++	++		+++	+++		8 x 7	+	+	8 x 8	++	++		
6	++	+++		14 x 13	+	+++	12 x 10	++	+++		+++	+++		7 x 6	±	+++	9 x 8	++	++		+++	+++		8 x 7	+	+	8 x 8	++	++		
8	++	+++		13 x 12	+	+++	10 x 10	++	+++		+++	+++		13 x 13	±	+++	14 x 13	+++	+++		+++	+++		15 x 13	+	+	11 x 10	++	++		
	++	+++		13 x 11	+	+++	10 x 8	++	+++		+++	+++		8 x 9	±	+++	13 x 11	+++	+++		+++	+++		15 x 13	+	+	12 x 10	++	++		
	++	+++		20 x 20	+	+++	15 x 10	++	+++		+++	+++									+++	+++									
	++	+++		20 x 18	+	+++	16 x 15	++	+++		+++	+++									+++	+++									

The letters P and M heading the columns stand respectively for polymorphonuclear leukocytes and large mononuclear phagocytes. The proportions of these types of infiltrating cells have been roughly estimated by four grades from ± to ++++. The figures in the columns labelled "Gross" indicate the size of the discolored area in the skin in millimeters.

bers of mononuclear phagocytes appeared, first in the surrounding tissues at some distance from the organisms and polymorphonuclear leukocytes, but fairly quickly condensing in a layer several cells thick just outside the neutrophils to form a loosely encapsulating wall. This process first became manifest on the 3rd day in a few only of the animals. By the 4th day, when hypersensitivity is well marked, it was never lacking. Figure 1 illustrates the localized abscess which regularly develops when living tubercle bacilli in doses from 0.1 to 2 mg. are injected intracutaneously into either sensitized or non-sensitized animals. This particular one was produced in a non-sensitized animal with a dose of 2 mg. and was excised on the 3rd day. Sensitization as judged by the tuberculin test had not yet developed. Figure 2 is a higher power of the corium surrounding the abscess and shows that even on the 3rd day the cellular reaction in this instance was almost purely polymorphonuclear. In contrast, Figures 3 and 4, likewise from the corium just outside the central abscess, but from a 24 hour old reaction in a pig sensitized 4 days before the intracutaneous reinfection, show very few polymorphonuclears, marked monocytic infiltration and definite proliferation of fibrous tissue. Reinfected lesions in the testicle developed in an essentially similar manner to those of the skin. Such lesions were produced in the guinea pigs recorded as Series II in the tabulated summary by the injection of 0.2 mg. of R<sub>1</sub> bacilli in the opposite testicle from that used for the primary infection. Figure 5 shows the exudate at 24 hours in a testicle that was infected 2 days after the first infection. The exudate consists almost wholly of infiltrated polymorphonuclears and mononuclear cells are entirely lacking. Figure 6, made again from a 24 hour lesion in a testicle but one where reinfection occurred 4 days after the first infection, shows large areas of tissue densely infiltrated with mononuclear leukocytes.

A comparison of reinfected lesions with primary infections, when interpreted in the light of our previous studies of the mononuclear character of the cellular response to tuberculin hypersensitivity, clearly showed a change in the character of the reaction on the 3rd day. At this time the reaction to tuberculin was not usually clearly positive. Probably the non-resorbability of the 'bacilli themselves makes them a more sensitive reagent for determining the first trace of hypersensitivity than tuberculin.

## THE PRIMARY INFECTION

The development of the lesions at the sites of the massive primary infections in the testicles, and for that matter in the soft tissues of the groin in Series II, proved remarkably uniform. During the relatively short periods over which the animals were observed, no noticeable difference was seen between the lesions produced by the  $R_1$  and the virulent strain. After 24 hours the bacilli were found enclosed in an abundant exudate consisting entirely of polymorphonuclear leukocytes (Fig. 7), while the surrounding tissue showed marked hyperemia and edema. At 48 hours distinct changes could be seen in the fibroblasts; they appeared much larger and more prominent, the nuclei stained more intensely, and the cytoplasm had increased in volume. By 72 hours distinct progression of the lesion had occurred (Figs. 8 and 11). Mononuclear phagocytes were appearing in abundance, the connective tissue cells had clearly multiplied, and occasional mitotic figures were encountered. By the 4th day a thick wall of mononuclears and young fibroblasts surrounded and encapsulated the abscesses. Mononuclears began to penetrate the abscess and to replace the polymorphonuclears. By the 5th day the granulation tissue had extended far from the site of injection and formed wide bands between the atrophying parenchymal tubules (Fig. 9). The lesions continued to advance until the 8th day, the last one studied, with progressive replacement of the abscesses with tuberculous granulation tissue. Only on the last 2 days could the development of "epithelioid cells" from the mononuclears be made out.

Minor variations in this pattern were observed. In Series II, for instance, there was a distinctly greater connective tissue reaction on the 2nd day than in any of the other sets of animals. In the group where one of the primary sites of infection was the groin, an area of hemorrhage 30 mm. in diameter appeared on the 3rd day. This did not, however, appear to modify the usual progress toward encapsulation of the lesion.

## SUMMARY OF EXPERIMENTAL EVIDENCE

The experimental evidence gathered from study of the tuberculin reactions and the reinfections may be summarized as follows: At a point not later than the 4th day, sometimes even on the 3rd



day, the reaction of the animals to tuberculin — usually more evidently to tubercle bacilli themselves — is clearly different in character from that observed in uninfected animals or in the first 2 days after the primary infection. It is quicker, far more intense in proportion to the dosage used, and microscopically can be shown to have the predominantly mononuclear character previously described<sup>1</sup> as characteristic of the delayed type of hypersensitivity. Even at this early stage the animals are clearly developing generalized tuberculin sensitivity.

At approximately the same time period — regularly on the 4th day, usually in incipient form on the 3rd day — a distinct change in the character of the primary lesion can be demonstrated. Whereas the reaction of the first 24 to 48 hours shows only congestion and serous exudation with marked accumulation of polymorphonuclear leukocytes — a reaction indistinguishable from that which might be produced by any pyogenic organism — beginning sometimes on the 3rd day and becoming well marked on the 4th, a second type of reaction has become obvious. Collections of large mononuclear phagocytes begin to appear as cuffs about the blood vessels and nerves, to infiltrate the stroma first at some distance from organisms and leukocytes but gradually to condense about them to form a wall several cells thick. At about this time the connective tissue cells begin to appear swollen and hyperchromatic, and mitotic figures begin to appear in abundance in them. In another 24 hours collagen begins to be laid down and true encapsulation is initiated. The parallelism between the appearance of demonstrable hypersensitivity and the alteration of the character of primary lesion are closer when both inoculations are made into similar tissues. When one injection is made into the peritoneal cavity and the other into the skin, for instance, noticeable differences in the rate of development of the lesions are apparent. The complications introduced by the absorption of large amounts of fluid exudate might readily explain the discrepancies however.

### DISCUSSION

Under the conditions of the experiments that have been described it has been shown that a very close parallelism exists between the appearance of demonstrable tuberculin sensitiveness and the shift from a purulent to a granulomatous infiltration of the

tissues. More exact determination of the time factors seems scarcely feasible when one reflects that a faint skin reaction of the delayed type can only be read 24 hours after it is performed — an interval during which it is fair to assume that the degree of hypersensitivity has been progressively and rapidly increasing. The discrepancies observed, therefore, seem to fall within the limits of error of the method and it is not unreasonable to conclude that hypersensitivity and alteration in the character of the histological response — an alteration which for the first time begins to suggest the typical granulomatous lesion — appear simultaneously.

It is realized, of course, that the massive infections used in these experiments have no counterpart in spontaneous tuberculosis of man or beast, but purposeful exaggeration of natural phenomena is one of the most useful methods of studying them and has been used by choice or necessity in most of the experimental work on the disease. A negative result from our experiments would have been strong evidence against the validity of our thesis. The result obtained is of value as strongly suggestive confirmatory evidence, but does not of itself constitute proof of similar time relationships under conditions of minimal infection. With the usual "spontaneous" infection of man or animal it is certain that weeks, probable that months may pass before generalized tuberculin sensitivity develops.

Obviously, to maintain our thesis we must fall back on the theory of local hypersensitiveness, always a favorite theme of speculation but except in special instances tantalizingly difficult to demonstrate experimentally.

Certain observations of Stewart are of great interest in this regard. In a study<sup>8</sup> of the histology of tuberculous infections of the testicle in guinea pigs rendered allergic with preliminary doses of heat-killed tubercle bacilli he noticed that attempts at healing — judged primarily on the basis of fibroblastic proliferation — became evident several days before generalized hypersensitivity could be recognized by skin tests. A probable explanation for this seemed to him to be the local development of allergy before it became generally detectable. In a second investigation<sup>9</sup> he was able to demonstrate this by the injection of tuberculin directly into the infected testis. A reaction characterized by extensive necrosis of the tubular cells, and an acute inflammatory reaction with hem-

orrhage and fibrin deposit appeared regularly when the injection was made on the 3rd day and became more marked on subsequent days. It was not until the 11th day that he observed positive reactions from tuberculin injections into the other uninfected testicle or in the skin. The time relationships of slight reactions on the 3rd day, well marked ones from the 4th day onward, are remarkably close to those we have demonstrated by a different technique.

Other forms of localized hypersensitivity, less directly applicable to tuberculosis but significant because of the more perfect control which is possible, are well known. The cutaneous drug-idiosyncracies, for instance, are known frequently to remain limited to the segment of skin originally exposed. Even protein sensitizations may remain localized to the treated area, as in the experiments of Redfern<sup>10</sup> and of Simon and Rackemann.<sup>11</sup> Not only sensitization but immunization, as represented by the presence of antibodies, has been shown on occasion to develop locally or to remain localized. Smith, Orcutt and Little,<sup>12</sup> for instance, showed that after inoculation of *Br. abortus* in one quarter of a cow's udder the antibodies appeared in highest concentration in the infected quarter. Recently McMaster and Hudack<sup>13</sup> observed that after injection of bacteria into the ear of mice agglutinin appeared first in the corresponding cervical lymph node where it could be detected for some time in higher concentration than in the blood serum or in extracts of other lymph nodes. In the light of our theory that bacterial allergy represents the first phase of the normal immune process, it is only reasonable to suppose that it also should appear locally earlier and in higher degree than general sensitivity.

It would be unwise to conclude without pointing out certain limitations to what we feel are justifiable deductions from our experiments. We are not attempting to maintain and do not believe it tenable that allergy is the only cause of mononuclear infiltration or of granulomatous reactions. The response to agar-agar or to lipid injections is too obvious evidence to the contrary. Nor would we wish to be understood as refusing to credit to the lipoids of the tubercle bacillus or to those developing in the course of caseation a rôle in the determination of the later stages of the cytology of the tubercle. The rôle of allergy in the early stages of tuberculosis lies in the greatly increased rapidity and disproportion-

tionate intensity with which the granulomatous response develops, a rôle that is certainly not without importance for the understanding of the disease.

### SUMMARY

A group of experiments designed to study the correlation of the early histological response to tuberculous infection with the development of allergy has been described and illustrated. In guinea pigs with massive primary infections of the peritoneal cavity or testicles, reinfectious lesions were produced on successive days and the animals simultaneously skin tested for tuberculin sensitivity. After the lapse of varying periods of time the primary and reinfectious lesions and the tuberculin reactions were compared histologically. Under the conditions employed and within the limits of error of the method, it was found that the first appearance of significant numbers of large mononuclear cells and the development of detectable tuberculin sensitivity occurred simultaneously between the 3rd and 4th days after the primary infection. The significance of these observations in relation to the authors' previously advanced hypothesis that bacterial allergy is responsible for the early granulomatous reaction of the host has been discussed.

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## DESCRIPTION OF PLATES

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### PLATE 133

- FIG. 1. The abscess produced by the intracutaneous injection of 2 mg. of  $R_1$  tubercle bacilli in a previously normal guinea pig. It was excised on the 3rd day after infection.  $\times 20$ .
- FIG. 2. A field from the corium at a little distance from the central abscess in this same case. The inflammatory response still consists entirely of polymorphonuclear leukocytes.  $\times 100$ .
- FIG. 3. A field from the corium bordering the central abscess produced by the intracutaneous injection of 0.1 mg. of  $R_1$  tubercle bacilli. The guinea pig had been primarily infected 4 days earlier with 10 mg. of  $R_1$  bacilli in the testicle, and the skin lesion was excised at the end of 24 hours. It shows rare polymorphonuclears, numerous monocytes and an increase in fibroblasts.  $\times 100$ .
- FIG. 4. The same.  $\times 300$ .
- FIG. 5. Exudate in the testicle some distance from the central abscess 24 hours after the injection of 0.1 mg.  $R_1$  bacilli in a guinea pig primarily infected 2 days before with 10 mg. of tubercle bacilli in the opposite testicle. A simultaneous tuberculin test was negative. The great predominance of polymorphonuclears and the paucity of mononuclears are evident.  $\times 250$ .
- FIG. 6. A corresponding field from a 24 hour old testicular lesion of a slightly allergic animal in which the reinfectious lesion was produced 4 days after the primary infection. The reaction at this spot is almost wholly mononuclear.  $\times 250$ .



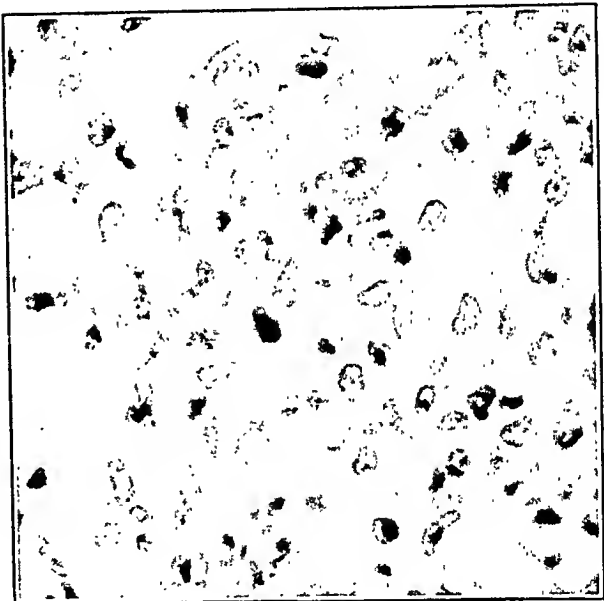
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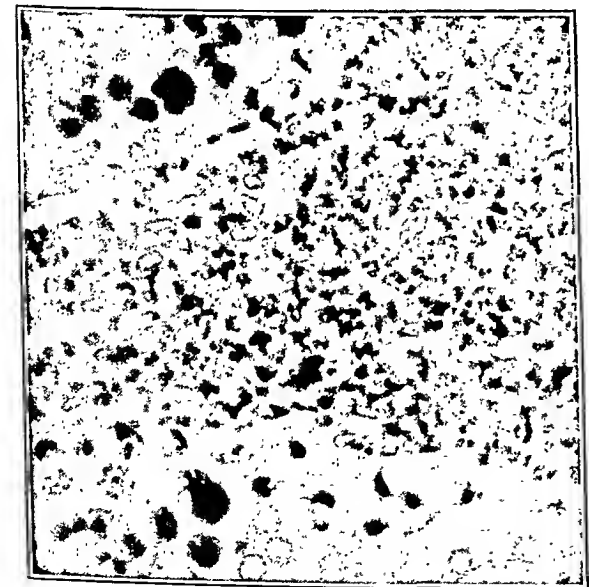
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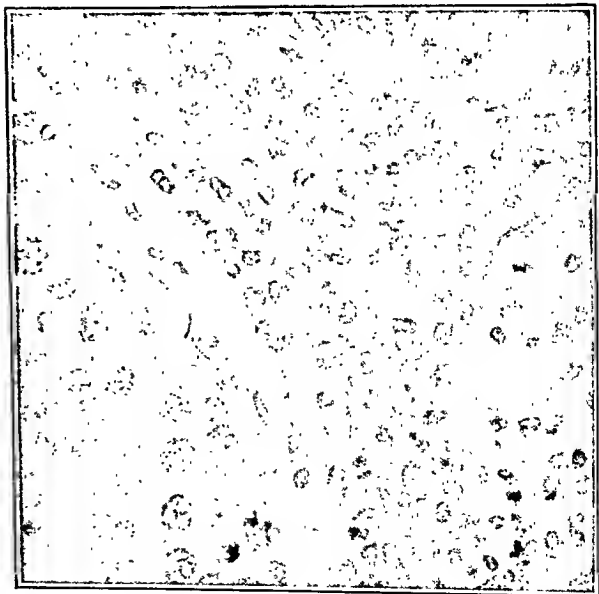
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PLATE 134

FIGS. 7, 8, 9 and 10 show primary lesions of the testicle produced by infection with 5 mg. of R<sub>1</sub> tubercle bacilli. They are respectively 1, 3, 5 and 8 days old.

Fig. 7. At 1 day a poorly defined abscess only is apparent.

Fig. 8. At 3 days a narrow rim of mononuclear infiltration and granulation tissue is evident.

Fig. 9. At 5 days the granulomatous encapsulation is broad and extends in narrow bands out into the surrounding parenchyma.

Fig. 10. At 8 days the abscess has been completely replaced by the granulomatous mass which occupies nearly the entire testicle.  $\times 6$ .

FIG. 11. A portion of the reacting tissue outside the abscess from the same testicle as Fig. 8, a 3 day lesion. In this area the reaction consists almost solely of mononuclear phagocytes.  $\times 250$ .

FIG. 12. The peritoneum of an intraperitoneally infected pig which shows the hemorrhagic reactions described in the text. In this area marked mononuclear infiltration is present.  $\times 100$ .



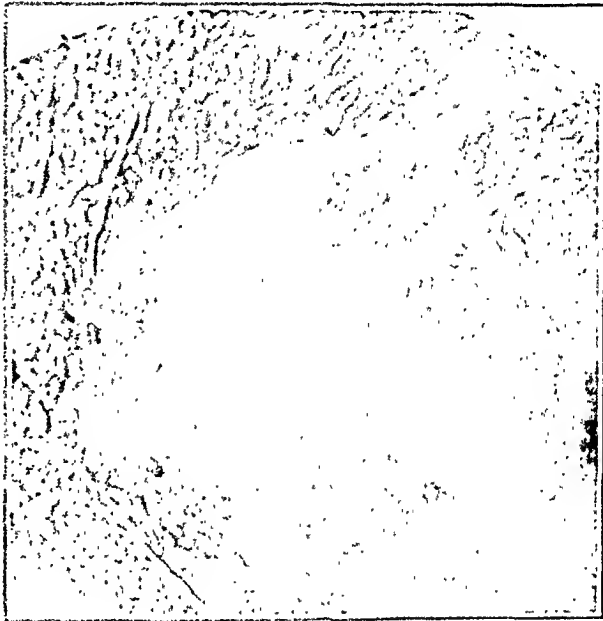
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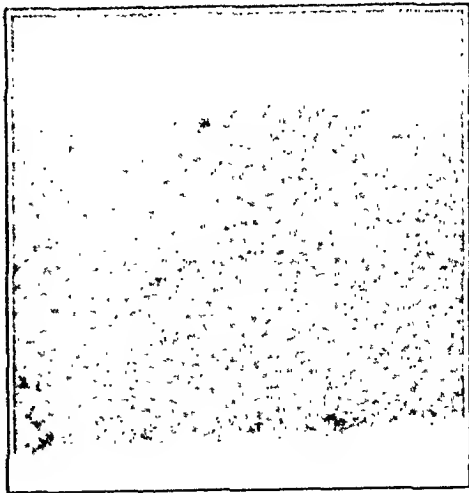
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# ENCEPHALITIS AND MENINGITIS IN THE CHICK EMBRYO FOLLOWING INOCULATION OF THE CHORIO-ALLANTOIC MEMBRANE WITH *H. INFLUENZAE* \*

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The use of the embryo chick for the study of bacterial infection was suggested by Goodpasture<sup>1</sup> in 1933. The report of Goodpasture and Anderson<sup>2</sup> on bacterial invasion of the chorio-allantoic membrane indicated that this method could be applied to the study of several types of microorganisms. The investigation of Buddingh and Polk<sup>3</sup> on meningococcus infection of the chick embryo further demonstrated its value for the study of the pathogenesis of this infection and suggested the use of this method for the analysis of the immunity factors in infection.

This report concerns an investigation of *Haemophilus influenzae* infection, using in general the methods and approach indicated by these workers. The use of the chick embryo has been extended to a comparative study of the pathogenesis of infection with various strains of *H. influenzae*, and of the effect of successive transfers through this host on the microorganisms and the infection which they produce.

## EXPERIMENTAL

### *Sources of Organisms*

Eight strains of *H. influenzae* were investigated:† Six had been isolated from autopsy material within the 48 hours previous to

\* Aided by grants from the Josiah Macy, Jr. Foundation and the Division of Medical Sciences, Rockefeller Foundation.

† The organisms were obtained from the following sources: The number refers to the autopsy, 1937, Vanderbilt Hospital. 14 = 48 hour ascitic broth culture of spinal fluid from a patient who later died of meningitis; 15 = pleural exudate obtained at autopsy 1 hour after death from *H. influenzae* pneumonia; 16 = 24 hour blood agar culture of exudate from a lung showing also *Str. viridans* and a hemolytic staphylococcus obtained at autopsy 3 hours postmortem; 32P = 24 hour blood agar culture of pleural exudate from an autopsy 5 hours after death from pneumonia and meningitis; 32M and 32MC = meningeal exudate from the same autopsy; 37 = meningeal exudate obtained at autopsy 5 hours postmortem; 55 = 24 hour blood agar culture of meningeal exudate from an autopsy 6 hours after death; and P = strain furnished by Dr. Caroline Chandler, isolated from a spinal fluid in 1934 and cultured in defibrinated rabbits' blood.

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their inoculation onto the egg. Of these, Nos. 15 and 32P were from pleural exudate, 16 from a pneumonic lung, and 32 (M and MC), 37 and 55 were from meningeal exudates. Two strains were from the spinal fluids of patients; 14 isolated 72 hours previous to inoculation, and P isolated in 1934. The latter strain was supplied by Dr. Caroline Chandler.

### *Characteristics of the Organisms Before Inoculation*

All strains were Gram-negative bacilli, which reduced nitrates, fermented dextrose, and required both the X and V factors for growth, as determined by their failure to grow in either autoclaved Levinthal broth or yeast extract broth. In determining the cultural, biochemical and immunological characteristics, and the pathogenicity of each strain, the reports of Pittman<sup>4</sup> and of Fothergill and Chandler<sup>5</sup> were largely followed.

With the exception of 16, all strains produced on Levinthal agar plates the giant iridescent colony typical of the S type. Strain 16 produced a giant colony similar to the S type in all respects except that it was never distinctly iridescent. Strains 32P and 32MC fermented galactose slightly. Strains 14, 15, 16, 32M and P fermented xylose slightly. No gas was produced in any of the eight sugars used.<sup>4</sup> Indol was produced by all with the exception of Strain 16.

Agglutination tests performed by the ordinary method with polyvalent horse serum gave unquestionable agglutinations only in serum dilutions 1:20, and occasionally in dilutions of 1:40 and 1:80, against all strains except 32MC. The latter gave definite agglutinations in serum dilutions through 1:1280. Clearer results were obtained by the thread reaction<sup>5</sup>; there was a sharp endpoint with all strains in serum dilutions varying between 1:320 and 1:1280. A positive precipitative reaction was obtained against polyvalent horse serum through 1:4 dilutions of the filtrate from the cultures of Strains 14, 32P and 37; through a 1:8 dilution of filtrate from 15, 32MC and 32M; and a 1:32 dilution of P filtrate. No definite precipitative reaction was obtained with Strain 16 filtrate.

The pathogenicity of each of these strains for mice and the reactions produced by the intradermal injection of rabbits were comparable with those reported for S strains of the organisms.<sup>4</sup> Death of mice was produced within 24 hours following the intra-

peritoneal injection of 0.5 cc. of a 20 hour culture grown in an Erlenmeyer flask of Levinthal broth (2.5 per cent blood). One tenth cc. of this culture introduced superficially into a rabbit's skin produced within 24 hours an elevated, definitely erythematous area about 2 cm. in diameter, which became indurated and remained from 1 to 2 weeks.

### *Experimental Procedure*

The chorio-allantoic membranes of chick embryos were exposed according to the coverslip method described by Goodpasture and Buddingh.<sup>6</sup> Series 14, 15 and 16 were each started by inoculating the membranes of 6 11 day eggs with a platinum loopful of a 24 hour Levinthal agar culture of the organisms. Similarly, for Series 32M meningeal exudate was directly inoculated, and for series P a 24 hour culture in defibrinated rabbit's blood was used. After inoculation the eggs were returned to the incubator.

Twenty-four hours later smears were made of exudates removed from the membranes with a platinum loop. These smears were stained by Wright's or Gram's method. A succeeding generation of a strain was started by transferring exudate from that membrane of the preceding generation which showed by smear the most profuse growth. A culture on a Levinthal agar plate was also made from this exudate. If available, 6 11 day eggs were used for each generation; sometimes fewer or older embryos had to be used. Occasionally, on account of fluctuations in the supply of fertile eggs, the interval between generations was greater than 24 hours; the greatest interval was 6 days.

In order to study the pathological changes produced by these strains originally, embryos were sacrificed at different stages of infection. An attempt was made to obtain a representative for each 24 hours postinoculation of each strain. As the survival period of the embryos of the different generations and series varied, and since the number in each generation was relatively small, several generations had to be drawn upon in order to obtain a complete group. The number in each group was limited by the duration of survival after infection, which varied from 4 to 10 days. These embryos and their chorio-allantoic membranes were fixed in Zenker's fluid with 10 per cent acetic acid. After removing the legs and wings, transverse sections of the entire fixed embryos were cut

and sections were stained by the hematoxylin-eosin and Giemsa methods.

With the strains later obtained the embryos for microscopic study were all taken from the same generation, for which 12 to 25 11 day embryos were inoculated. This was done at the 5th generation of 32*M*. Subsequently an attempt was made to give the same infecting dose to each member of the large generation to be used for sections. In Series 32*MC* and 32*P* the organisms from a 24 hour Levinthal agar subculture were suspended in sterile chick amniotic fluid. A drop of this suspension was dropped from a capillary pipette onto each membrane. Likewise, for the embryos for section, the third generation of 16*B* and the second of 55 were inoculated with a mixture of the membranal exudate and allantoic fluid of the egg chosen from the preceding generation. In Series 37 the embryos for study were inoculated with a loopful of the meningeal exudate.

Routine smears of the exudate from the membrane, and cultures on Levinthal agar from the membrane and heart's blood, were made at the time of sacrifice of each embryo of these larger generations.

Strains 37 and 55 were not transmitted through further generations. All other strains were transferred through 20 generations of chorio-allantoic membranes. The only contamination in the transfer series was of Strain 14 with staphylococcus in the 12th generation. This mixed growth was transferred through the 13th and 14th generations; the 15th generation was inoculated from a typical S colony of a 16 hour culture of the exudate of Generation 14 on Levinthal agar. With the exception of Strain 16, a sufficient number of the embryos survived the infection long enough to make the maintenance of the strains through successive generations and the investigation into the pathological process easily possible. During one of the periods when no fertile eggs over 10 days old were available, all the embryos of Series 16 died; the strain from a culture of the last (9th) generation was maintained for 4 days by daily transfers on Levinthal media. A new series (16*B*) was then started by inoculating 6 11 day embryos from this 4th Levinthal agar culture of the 9th egg generation of 16.

At the 20th generation another series of embryos was taken from each strain for microscopic study of the infection at 24 hour

intervals after inoculation. For this purpose from 12 to 25 11 day chorio-allantoic membranes were inoculated with a drop of the suspension of the membranal exudate in the allantoic fluid from the chosen 19th generation egg. To confirm initial infection Levinthal agar plate cultures were made from the exudate of each 20th generation membrane 24 hours after inoculation; all negative eggs were discarded. The sacrificed embryos were treated as described for the earlier generation.

A record of each series was kept according to the following chart, which is an elaboration of that used by Buddingh and Polk.

	DAY 1 24 HRS	DAY 2 48 HRS	DAY 3 72 HRS	DAY 4 96 HRS	DAY 5	DAY 6	DAY 7	DAY 8	DAY 9	DAY 10	DAY 11
P I 1-30-37 11 DAY EMB.	□○○ ⊗○○	○○○ ○	□□⊗ ○	○○ ∅	□○	□○	⊗⊗				
P II 2-1-37 12 DAY EMB.	○○○ ⊗○○	⊗○○ ○	○ ∅	□	○		□	⊗			
P III 2-3-37 11 DAY EMB.	○○○ ○○○	○○○ ○○○	○○□ ○○○		○○□ ⊗⊗⊗	○○□ ⊗	□	□		⊗	
P IV 2-9-37 11 DAY EMB.	○○○ ○○○	⊗⊗○ ⊗⊗⊗		● ●							
P V 2-11-37 10 DAY EMB.	○○⊗ ●○○	○				●	⊗	●			●

EMB - EMBRYOS

○ EMBRYO SHOWING BACTERIA ON THE MEMBRANE.

□ EMBRYO SHOWING NO BACTERIA ON THE MEMBRANE.

⊗ EMBRYO SHOWING CONTAMINATION OF THE MEMBRANE.

∅∅ SACRIFICED EMBRYOS.

● DEAD EMBRYO.

CHART 1. Each horizontal row, divided into 24 hour intervals, represents 1 generation of inoculated embryos. The strain, generation number, date of inoculation and age of the embryos used, are noted in the first square. The arrow indicates the embryo from which the succeeding generation was inoculated.

The culture, biochemical and immunological characteristics and the pathogenicity were determined for each of the 19th generation strains used to inoculate the 20th generation.

Cultures of each original strain were carried through defibrinated rabbit's blood and the incubation and time of transfer were the same as for the egg series. These cultures were studied as controls.

*Effect on the Characteristics of H. influenzae of Transference  
Through 20 Generations on the Chorio-Allantoic Membrane  
of the Chick Embryo*

There was no change in the toxicity of the strains to mice or in the skin reaction of rabbits. There was no alteration of the agglutination by polyvalent serum with Strains 16B, 32M, 32MC or P. Strains 32P and 15 originally gave an ordinary agglutination reaction in serum dilution through 1:40; after the 20th egg generation these strains gave definite agglutinations through serum dilutions of 1:5120; the control 20th generation rabbits' blood cultures did not show an increase in agglutinability. The end-point of the thread reaction was in the next serum dilution above that shown originally for each strain. The precipitative reaction end-point was consistently in the next lower dilution of the filtrate than it was originally. Morphologically the organisms were indistinguishable. Except for a marked diminution in iridescence in the 8th and 9th generations of Strain 14, the colonies on Levinthal agar plates throughout the 20 generations of all strains showed the original characteristics.

*Studies of the Inoculated Chorio-Allantoic Membrane*

The gross lesions on the membrane varied greatly, even within the same generation, and no characteristic appearance for any one strain was noted. Usually within 24 hours after inoculation the membrane became edematous and moist. The process progressed during the following 24 to 48 hours (Fig. 1), then regressed, leaving finally a dry, slightly thickened membrane at the end of 6 days. One or more areas of pale yellow, moderately thick exudate were found; during the edematous stage these areas frequently appeared as slight depressions and remained as small crusted areas on the dry membrane. Frequently, although at the time of mechanically exposing the membrane no trauma was evident, 24 hours after inoculation the outline of the square window could be traced as lines of purulent exudate on the membrane. Occasionally an increased purulent exudation produced a heaped up mass which became a crusted nodule as the membrane dried.

Smears made of the material obtained by scraping the membrane with a platinum loop and stained with Wright's stain showed an

inconstant picture. The exudate usually consisted of red blood cells, mononuclear cells, polymorphonuclear leukocytes and bacteria. The relative numbers of these varied in the exudate of the same series, even in the same generations, and there was nothing by which the strain could be differentiated. With very few exceptions mononuclear cells predominated. Lymphocytes were rarely found; the majority of the mononuclear cells were monocytes with the characteristic purple loose nucleus and pale blue cytoplasm, which in the early stages of infection contained large eosinophilic masses. These masses were also found free and probably were hyaline bodies from ruptured degenerating epithelial cells. Another quite similar type of granulated cell, which by some methods showed a poorly stained nucleus, suggested desquamated degenerating epithelial cells. There were a few deeply staining, compact mononuclear cells of variable size, sometimes with pale blue nucleoli and usually containing a few, large, purplish red granules which were classified as myelocytes. The polymorphonuclear cells contained large fusiform eosinophilic granules.

All strains of bacteria showed some pleomorphism with a tendency to greater variation in morphology as the infection persisted. The degree of degeneration of the bacteria varied inconstantly; sometimes even within 24 hours most of the bacteria appeared as oval masses which stained only peripherally (Fig. 2); in other instances even at 96 hours the organisms were well preserved, regular and deeply staining. All strains showed intracellular organisms in most of the smears; this was less notable, however, with the stock strain *P*. In the early stages of infection (24 to 48 hours) cells were found distended with a mass of deeply staining bacilli, the nucleus being flattened against the periphery (Fig. 3). Such cells could not always be identified but the majority were monocytes. Other monocytes contained fewer bacteria which sometimes were thick and pale staining; occasionally a small deeply staining mass of bacteria was separated from the mass of blue cytoplasm by a clear zone. In the latter and in disrupted cells of the first described type tiny eosinophilic rods were found. As the infection persisted fewer monocytes containing bacteria were found. Of these, the relative number containing a few, large bacillary forms increased; in the others the intracellular masses of bacteria became predominantly eosinophilic. Usually by the



5th day of infection only an occasional cell containing a few bacilli was found. In those instances in which the bacteria showed degeneration the cells also were vacuolated, poorly preserved, and intracellular organisms showed as irregular blue staining masses (Fig. 2).

Smears from the earlier generations were indistinguishable from the 20th generation smears.

Sections of the membranes showed a more constant and progressive picture. At the end of 24 hours after inoculation there were localized lesions characterized by a superficial exudate overlying an area of ectodermal hyperplasia. The superficial ectodermal cells were vacuolated and at times contained large eosinophilic masses. Beneath this area hyperplastic fibroblasts separated the vascular zone from the epithelium. The vessels of the vascular zone were hyperemic and frequently there were areas of hemorrhage in this region. Occasionally a thrombosed capillary was seen. The underlying endodermal cells were hyperplastic and a columnar layer was beginning to differentiate. Forty-eight hours after inoculation (Fig. 6) the zone of fibroblastic growth had widened and become vascularized, the epithelium above it was stratified, and the superficial cells showed hyalinization which fused with the mass of exudate and bacteria. In some membranes there were scattered mononuclear cells in the adjacent mesoderm. These cells had an intense, deep blue staining cytoplasm and nucleolus, and suggested a primitive wandering cell; a few intermediate forms suggested the mesoderm as the origin. Clusters of these cells were occasionally found about vessels. A few membranes showed scattered and perivascular polymorphonuclear leukocytes in the mesoderm. In the dislodged vascular zone there were islands of ectodermal cells. After 48 hours the zone of fibroblastic tissue widened and became less dense. By the 5th day, in some instances, the only evidence of the earlier lesion was a localized area of slightly thickened ectoderm and entoderm and, in the intervening vascular mesoderm, islands of epithelial cells arranged in a semicircle opening toward the ectoderm. In other membranes sometimes as early as the 3rd day, but usually about the 5th day, there appeared an increased perivascular infiltration of polymorphonuclear leukocytes, "primitive cells" and monocytes. This reaction was most notable in, and in many membranes limited to, the area about the

initial lesion. In only a few instances was the epithelial layer of the membrane ulcerated, and only very rarely were there bacteria in the membrane.

### *Histopathological Studies of the Embryos*

One hundred and nineteen embryos of the series were studied. The most constant but probably non-specific finding was a perivascular infiltration found chiefly in the lungs, portal spaces of the liver, skin, pharynx and connective tissue. In a few of the 72 hour infections and in an occasional 96 hour infection this infiltration was present. The infiltrating cells were the "primitive type" and eosinophilic polymorphonuclear leukocytes. In most 5th and 6th day infections there was a perivascular infiltration of variable intensity with eosinophilic polymorphonuclear leukocytes, monocytes and a few lymphocytes. This reaction was less notable in the succeeding days, but in 1 embryo 10 days after inoculation it was present. It was not found in the few hatched chickens that were sectioned. The degree and type of perivascular infiltration in the embryo was similar to that found in the membrane in the region of the initial lesion. Nearly all embryos 4 days or more post-inoculation showed scattered polymorphonuclear leukocytes in the cerebellar meninges. These were rarely seen in uninoculated embryos.

More significant lesions were found in the first 4 embryos sacrificed in the 3rd generation of 16B series, in 1 embryo of 32M series, and one of 32MC series. The embryos of the 16B series will be described first.

In the brain of the embryo sacrificed 24 hours after inoculation a few small areas of early necrosis were found. In the tissue between the skin and pineal body, in which meninges, dura and connective tissue are not demarcated and in which there is no bone, areas of hemorrhage were found (Fig. 4). In a few of the capillaries in these areas masses of necrotic blood cells were seen and several definite bacilli (Fig. 5) were found both extracellularly and within monocytes. The intracellular forms were shorter and thicker. Heart blood culture was negative.

In the brain of the 48 hour specimen several areas of hemorrhage were found (Figs. 7 and 8). Many of these red blood cells were necrotic and the included capillaries were occluded with necrotic

cells and degenerated endothelial cells. Throughout the brain the capillaries were conspicuously filled with and even appeared occluded by large mononuclear cells. Most of these mononuclear cells were monocytes of the usual type but many were large "primitive type" cells. In the ventricles there was an exudate of these mononuclear cells and polymorphonuclear leukocytes. The meninges and the tissue between the skin and the pineal body were edematous and slightly infiltrated with polymorphonuclear leukocytes and a few monocytes. Some of the monocytes contained degenerated red blood cells. Many of the capillaries in this area had the same appearance as those in the brain substance and in addition a perivascular infiltration with similar mononuclear cells. Scattered throughout the tissues in general in the body and quite prominent in the periportal spaces of the liver were small accumulations of the intense blue staining ("primitive type") monocytes. No bacteria could be positively identified in the sections but the blood culture was positive.

Apparently the above process had progressed to produce a remarkable picture 72 hours after inoculation of the membrane (Fig. 10). Large areas of the brain were necrotic and consisted of a loose mass of red blood cells, brain cells and leukocytes. These necrotic areas had ruptured into the ventricles, which were filled with débris, polymorphonuclear leukocytes, monocytes, red blood cells and numerous pleomorphic bacilli (Fig. 12). There was considerable perivascular infiltration of the meningeal vessels with both types of mononuclear cells and a few polymorphonuclear leukocytes. In the dura were areas densely packed with the intense blue staining mononuclear cells, a few of which contained eosinophilic granules. Small groups of similar cells were found perivascularly in the lungs, portal spaces of the liver, and occasionally in the connective tissue of the skin. In the thoracic and cervical spinal meninges (Fig. 11) an exudate of polymorphonuclear leukocytes and monocytes was found; no bacteria were identified in it. The heart blood culture of this embryo was negative.

The 96 hour specimen showed less extensive areas of necrosis in the brain than the 72 hour, but the process appeared to be essentially the same. The perivascular infiltration of the meningeal vessels was more intense. There was an exudate of polymorpho-

nuclear leukocytes and monocytes in the ventricles, spinal meninges and pericardium; no bacteria were identified positively. The blood culture was positive. All organs showed a perivascular infiltration with monocytes and polymorphonuclear leukocytes.

An embryo of the 1st generation of 32MC, which was sacrificed 72 hours after inoculation, showed a picture (Fig. 9) similar to that found in 16B at 72 hours, except that there was not the extensive rupture of necrotic brain tissue into the ventricles; the exudate in the ventricles was chiefly of large monocytes and no bacteria could be demonstrated. The blood culture was negative.

A 2nd embryo taken 5 days after inoculation of the third generation of 32M showed areas of hemorrhage and necrosis in the brain, an exudate of foamy mononuclear cells in the ventricles, and moderate perivascular infiltration with monocytes and polymorphonuclear cells of the dura, meninges and parenchymatous organs.

The exudate from the membranes of these embryos showed well preserved, numerous intracellular and extracellular organisms; the cells were well preserved and possibly less numerous than usual in 16. The membranes of the 72 hour (16B) (Fig. 6), 96 hour (16B) and 5 day (32M) were unusual histologically in that each showed a break in the ectoderm by which the bacteria-laden exudate was in communication with the mesoderm. Bacteria were found in the mesoderm in 32M free beneath the ectoderm, in a localized necrotic area completely walled off from the surrounding mesoderm by fibroblasts in 96 hours (16B), and in necrotic exudate in a thrombosed vessel in 72 hours (16B).

#### *Survival of the Organisms in the Chorio-Allantoic Membrane and the Blood Stream Invasion of the Embryo*

Heart blood cultures from the sacrificed embryos showed no constant time of invasion or of disappearance of organisms from the blood stream. Each strain gave at least 1 positive blood culture. No hatched chickens gave a positive blood culture. In all strains except P the membrane continued infected through the 4th day; several thereafter became sterile. The membrane of a hatching chicken of Series 15 was positive.

TABLE I

## Membrane and Heart's Blood Cultures of Different Series of Inoculated Embryos

Strain	Generation	Culture	24 hours	48 hours	72 hours	4 days	5 days	6 days	7 days	8 days	9 days	10 days
<i>r6B</i>	3	M HB	++ -	++ +	++ -	++ +	- -	+				
<i>32M</i>	5	M HB	++ -	++ -	++ +	++ -	++ -	++ -				
<i>32P</i>	1	M HB	++ -	++ +	++ +	++ -						
<i>32MC</i>	1	M HB	+++ +	+++ +	+++ -	- -		+				
<i>37</i>	1	M HB	++ -	++ -		++ +	- -					
<i>55</i>	2	M HB	+++ -	+++ -	+++ -	++ +	++ -					
<i>P</i>	20	M HB	++ -	++ +		- -	++ -	- -	- -	+		
<i>14</i>	20	M HB	++ +	+++ +	++ -	++ +	++ +	- +	+			
<i>15</i>	20	M HB	+++ +	+++ -	++ -	++ +	- -					
<i>16</i>	20	M HB	+++ +++	+++ +++	++ +	+++ -	++ -					
<i>32M</i>	20	M HB	+++ +++	+++ +	+++ -	++ -	++ -	++ -	- -		- -	+
<i>32P</i>	20	M HB	+++ -	+++ +		- +	+++ +	- -	+	++ -	- -	+
<i>32MC</i>	20	M HB	+++ +++	+++ +	++ +	+++ -	+++ -	+++ -	+++ +	- -	+	

HB = Heart blood culture on Levinthal agar.  
M = Membrane culture on Levinthal agar.  
+ to +++ = Relative number of colonies.  
- = Negative culture.

*Survival of the Embryos After Inoculation of the Chorio-  
Allantoic Membranes*

The survival period varied considerably for the same strain. After the first 3 generations of 16 the embryos of that series, with occasional exceptions, died within 48 hours; 1 of the embryos hatched. None of Series 14 survived the 9th day after inoculation, but many survived 5 days. Several of Series P and 14 survived 11 days after inoculation and 1 of each hatched. The embryos of Strains 32 had on the whole the longest survival period; 3 of the 32MC and 3 of the 32M hatched.

*Immunological Studies of the Blood of Chickens Hatched From  
the Inoculated Series*

These chickens were sacrificed and the serum used for agglutination tests against the strain of organisms inoculated on the membrane. Thread and ordinary agglutination reactions were negative. The precipitative reactions were negative.

## DISCUSSION

This work has been reported in detail in order to help further investigation in a relatively new field, rather than for any significance in itself. Now that it has been found by chance that a lesion can be produced by *H. influenzae*, which is in many respects a counterpart of meningitis and ependymitis in children (Fig. 13), although the lesion has not yet been reproduced at will, the factors determining this initial infection become of primary interest. The chick embryo offers unusual possibilities in the investigation of such a problem.

From this investigation no conclusions can be drawn concerning the rôle or relations of any of the variable factors — host resistance, host susceptibility, virulence and quantity of organisms, duration of infection or availability of the organisms to susceptible tissues — in the establishment of the pathological process. That there may be a variation in virulence which may be an important factor is suggested by the fact that the 4 significant embryos of 16B came from the same generation, whereas embryos from several other generations of 16 showed no encephalitis or meningitis. The strain used in starting 16B had all the characteristics, as de-

terminated by the usual methods, that the original strain and the 20th generation strain possessed. Our present methods of determining virulence, however, are admittedly inadequate. Only 1 embryo was sacrificed from the generation of 32*M* showing encephalitis. That the virulence of the organisms is the only determining factor is contradicted by the fact that the embryo taken 6 days after inoculation in the same generation of the 16*B* series, and the other 5 taken from the 32*MC* series, showed no evidence of encephalitis or meningitis. In the membranes of 3 out of the 6 significant embryos there was a break in the epithelium and bacteria were found within the membrane. That a source of continued infection is necessary and is obtained by some such process is suggested. However, the invasion of the membrane may be a reflection of the virulence of the organism or the general susceptibility of the host. In only 3 other membranes was a break in the epithelial barrier noted; these embryos were not remarkable. The numerous positive blood cultures in embryos showing no encephalitis eliminate a bacteremia as the sole determining factor. The reaction in the tissues between the skin and the brain in the early stages of infection (16*B* 24 hours and 16*B* 48 hours) may be of significance and immediately suggests the anatomical difference between infants, who are susceptible to influenza meningitis, and adults, who are not. Cultures of the amniotic fluid were not made and further investigation of this as a source of infection is indicated. Experiments with *H. pertussis* suggest that ready accessibility of the microorganism to the susceptible tissues is a very important factor, and that altering the route of inoculation may yield more constant infections.

The cause of the perivascular reaction in the later stages of infection of most of the embryos is not clear. No organisms were ever demonstrable in these areas. The early response with the intense blue staining mononuclears may represent a hyperplasia rather than an infiltration. According to the blood culture findings, the perivascular reaction does not depend on a continued bacteremia. The possibility of circulating toxins is to be considered. The continued bacterial growth in the initial lesion of the membrane in some instances might be a source of toxin production; however, the perivascular reaction was sometimes found when the membranes had become sterile. One embryo, which showed con-

siderable perivascular reaction and had a sterile culture of the blood and epithelial surface of the membrane, showed organisms adherent to the endoderm. Cultures of the allantoic fluid were not taken but growth in this extensive medium must be considered in explaining the perivascular reaction.

It is hoped that by redirected and further investigation infection of the brain and meninges can be consistently produced. When this is accomplished a comparative study of the various strains and the effect of successive transfers may be effectively undertaken. Also, and of more immediate significance, immunological studies can then be started.

### SUMMARY

A detailed account of the study of *H. influenzae* infection by the use of the chick embryo is given. In the course of the study a few of the embryos were found to have an encephalitis and meningitis. Investigations to determine the factors which establish this infection with *H. influenzae* and by which these lesions will be consistently produced are indicated.

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## DESCRIPTION OF PLATES

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### PLATE 135

- FIG. 1. Egg showing chorio-allantoic membrane with purulent exudate. *H. influenzae* 48 hours.
- FIG. 2. Membranal exudate. Degenerated cells and intracellular and extracellular bacilli (dark masses).  $\times 1600$ .
- FIG. 3. Membranal exudate. Well preserved intracellular and extracellular bacilli.  $\times 1600$ .
- FIG. 4. Hemorrhage and necrosis of blood cells.  $\times 550$ .
- FIG. 5. Lower right section of Fig. 4 enlarged to show bacilli.  $\times 1400$ .
- FIG. 6. Chorio-allantoic membrane; *H. influenzae* 48 hours.  $\times 55$ .

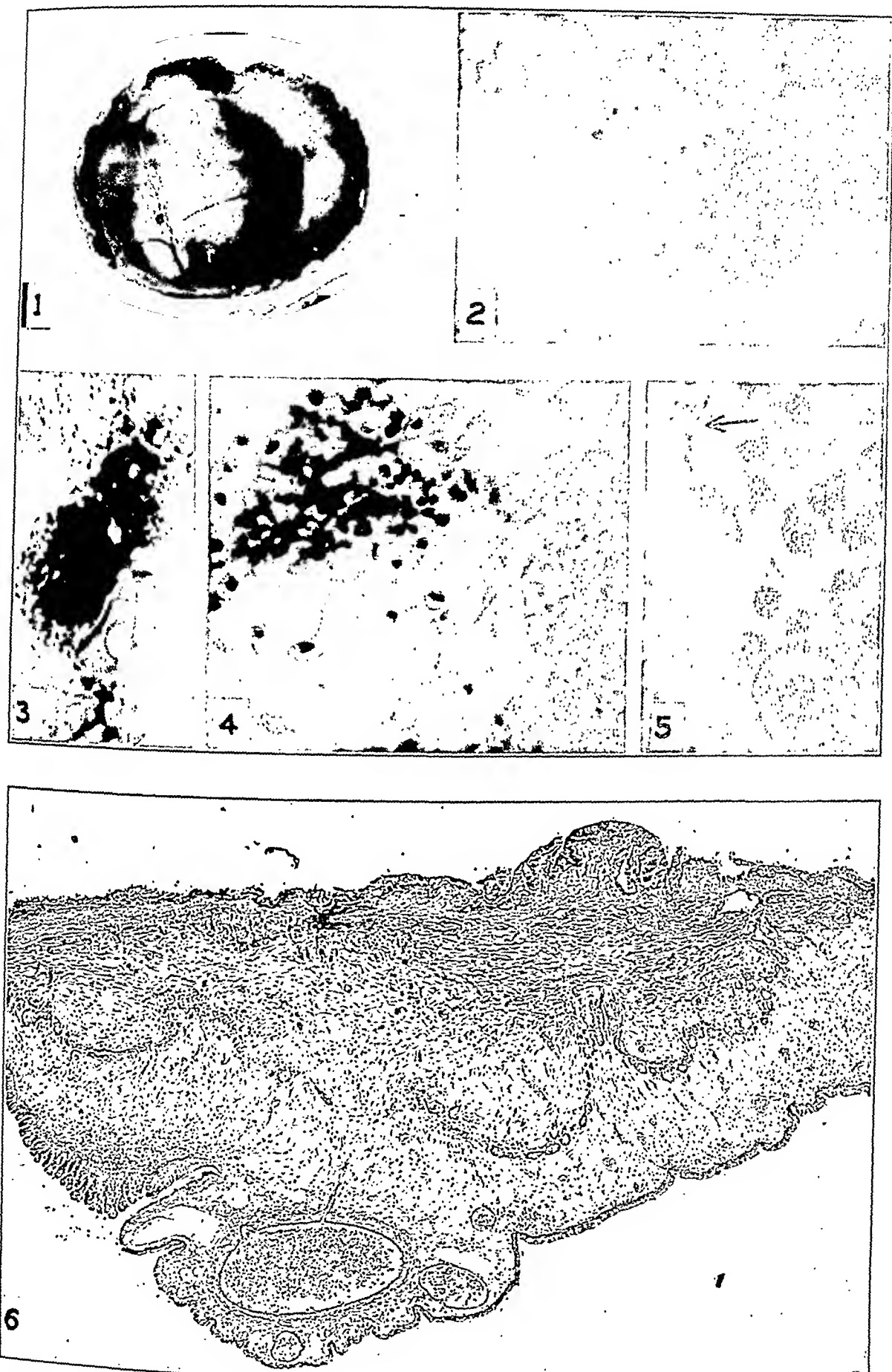
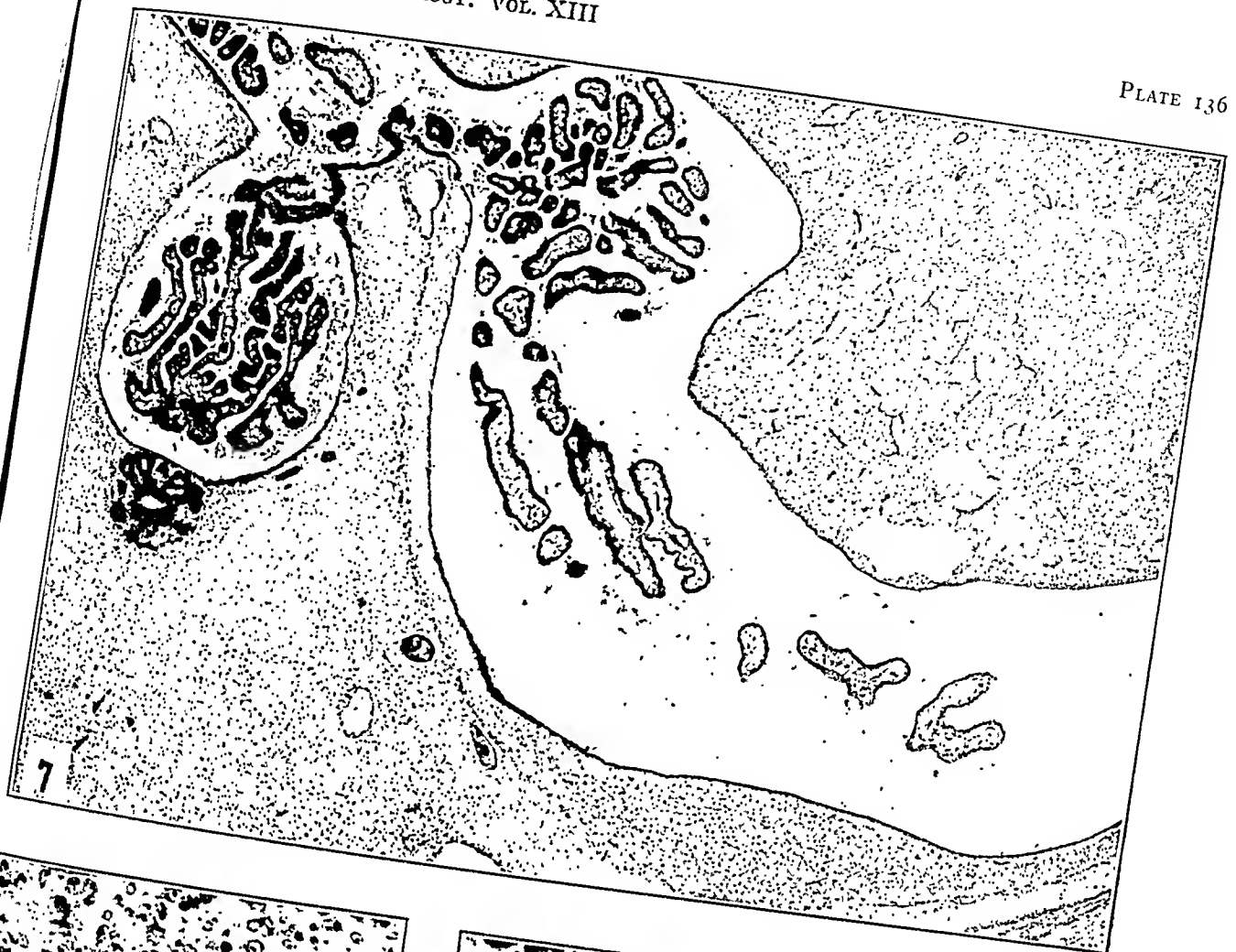


PLATE 136

- FIG. 7. Brain of chick embryo 48 hours postinoculation of membrane with *H. influenzae* 16B. Note area of hemorrhage in upper right corner, conspicuous capillaries in the brain represented by the dark branching structures, dilated ventricles containing cellular exudate and cellular infiltration of the connective tissue.  $\times 30$
- FIG. 8. Area of hemorrhage and necrosis of blood cells seen in Fig 7.  $\times 200$ .
- FIG. 9. Brain of embryo 72 hours postinoculation of membrane with *H. influenzae* 32MC.  $\times 44$ .



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Encephalitis and Meningitis in Chick Embryo

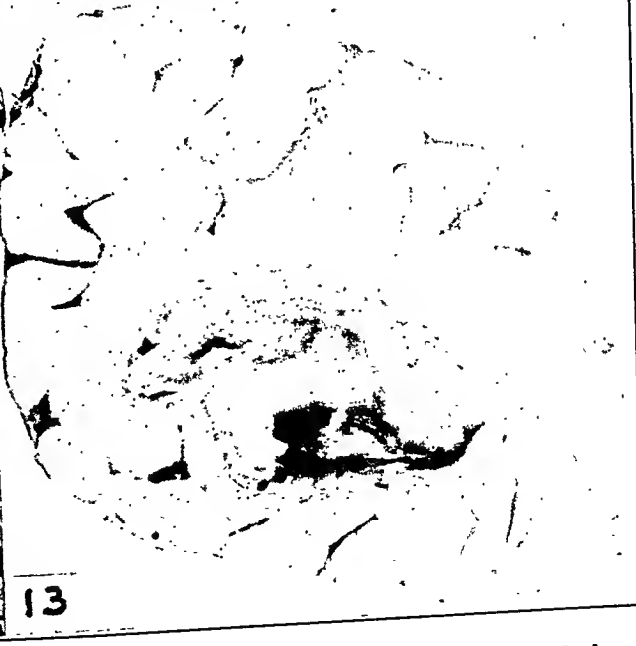
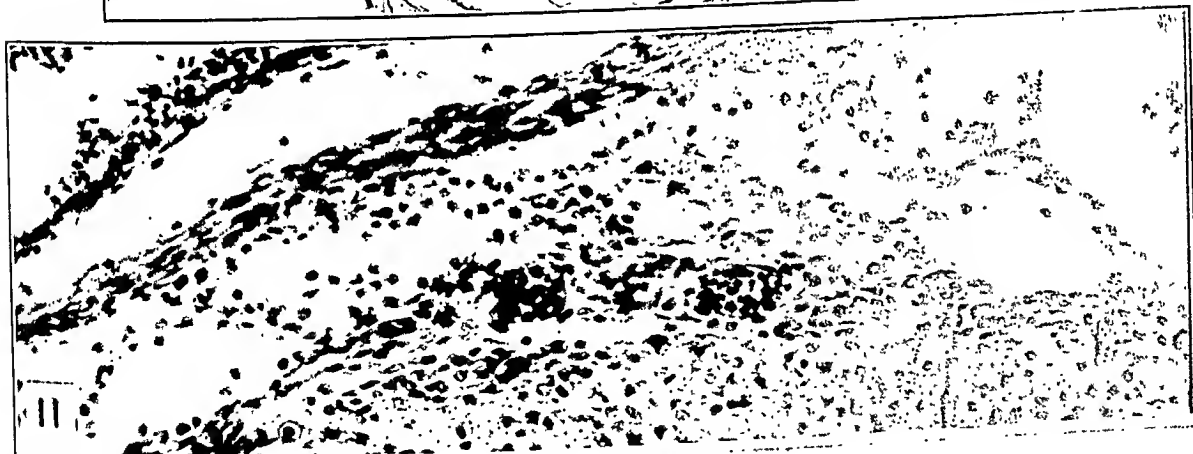
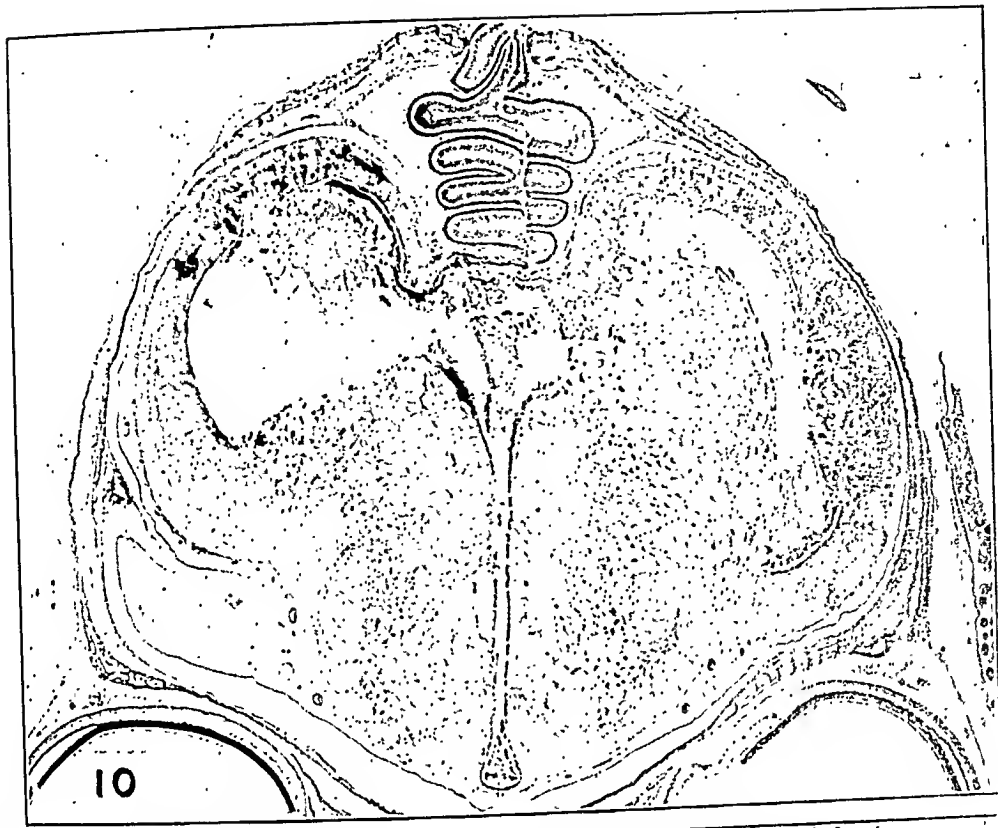
PLATE 137

FIG. 10. Brain of embryo 72 hours postinoculation of membrane with *H. influenzae* 16B.  $\times 9$ .

FIG. 11. Exudate in spinal meninges of chick embryo.  $\times 190$ .

FIG. 12. Exudate shown in ventricle in Fig. 10. Irregular black mass represents a clump of bacilli.  $\times 1200$ .

FIG. 13. Gross section of brain of a child 2 years of age showing suppurative ependymitis, encephalitis, meningitis and hydrocephalus.





# INFECTION OF CHICK EMBRYOS WITH *H. PERTUSSIS* REPRODUCING PULMONARY LESIONS OF WHOOPING COUGH\*

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The rôle of *Haemophilus pertussis* in the production of pulmonary lesions of human whooping cough has been the subject of much dispute. The following experiments throw some new light on this subject and indicate the method by which a more extensive study of *H. pertussis* infection in relation to the etiology and pathogenesis of whooping cough is being carried on.

As a preliminary to the study of experimental infection with *H. pertussis* the microscopic sections of 11 autopsy cases on record at Vanderbilt Hospital were reviewed.

In addition to the presence of Gram-negative bacilli between the cilia, the inflammatory reaction in and about the trachea, bronchi and bronchioles, and the bronchopneumonia, we were impressed by the presence of a lesion in the respiratory epithelium, especially that of the bronchi and bronchioles, which has attracted little or no attention.

Arnheim<sup>1</sup> mentioned desquamation of ciliated epithelium in whooping cough and described small bacilli on these cells. Mallory and Horner<sup>2</sup> gave a more detailed description of the peculiar relation of the bacilli to the ciliated epithelium in 3 cases, but stated that they could find no evidence of epithelial necrosis, nor did they describe any degenerative changes in these cells.

The studies of Feyrter<sup>3</sup> tend to confirm the impression of Posposchill that the lung is the seat of the essential lesions of whooping cough. In describing the respiratory epithelium he cites inflammatory infiltration, sometimes with miliary abscess formation, in the bronchial epithelial layer, even rupturing into the lumen and leaving small ulcers.

In our study of human material it appeared also that the severest lesions, in association with the bacilli situated in the characteristic interciliary position or on the partially deciliated border, were in

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the bronchi and bronchioles, and to a less extent in the trachea and the nasopharyngeal ciliated epithelium (studied in 1 case).

The inflammatory response to the presence of the bacilli is no doubt due to injury induced by these specific microorganisms, although the nature of that injury has not been revealed by previous work. In our cases the injury appears to be for the most part manifested in the appearance of the respiratory epithelial layer usually at points surmounted by a zone of interciliary bacilli. This injury is relatively mild but may induce necrosis. The cells most affected, as indicated by their cytology, are situated in the midzonal or basal layers of the epithelial covering of bronchi and bronchioles. The superficial ciliated cells are relatively intact. Degeneration and even necrosis of the deeper epithelial cells may be demonstrated, and the inflammatory reaction, polymorphonuclear leukocytes, macrophages (some of which appear to be derived from epithelium) and mitotic figures correspond in intensity to the degenerative lesion — that is to say, it is most pronounced in the midzonal and basal layer. The epithelium is in places elevated from its basement membrane by exudate, and miliary abscesses (foci of polymorphonuclears and macrophages) frequently are limited to the midzonal layer.

The situation of this chief injury and inflammatory reaction in the midzonal and basal layers of the bronchial epithelium is quite characteristic. The type of lesion suggests the action of a specific toxic agent. This type of lesion was not found in the cases of interstitial bronchopneumonia associated with influenza and measles which were examined. Whether or not the associated bronchopneumonia may be caused by *H. pertussis* is as yet not determined for the human disease.

It is evident that further study of the human lesion is necessary for the establishment of the pathogenesis of whooping cough, but there can be no doubt that a specific lesion exists.

It would be highly desirable to have at hand a suitable susceptible experimental animal in which a more thorough study of the etiology and pathogenesis of this important disease could be carried out. None of the usual laboratory animals, with the possible exception of some monkeys and the chimpanzee, appear to be susceptible to the infection.

Although most investigators seem to believe that *H. pertussis*

is the chief or sole inciting agent, there are others who think that an undetermined virus may be an essential preliminary invader.

In the investigation of *H. pertussis* infection in chick embryos to be described, we believe the essential lesion of whooping cough has been reproduced, and it is hoped that the method employed may serve as a means of throwing additional light on the etiology, pathogenesis and immunology of this specific infection.

### EXPERIMENTAL

The first part of the experimental investigation of *H. pertussis* was started at the same time and undertaken in the same manner as that of *H. influenzae* reported in the present issue of this journal.

Only one strain of *H. pertussis* was studied, and no clinical cases or autopsy material were available during the investigation. The strain used, isolated by Dr. John Toomey, Oct. 24, 1936 and kindly sent to us by him, had been transferred a number of times on Bordet-Gengou medium containing 25 per cent human blood, and then several times on Difco Bordet-Gengou agar with 25 per cent defibrinated rabbits' blood. The latter medium was used throughout the experiment.

This strain of *H. pertussis* had the following characteristics: morphologically it was a small Gram-negative bacillus which grew slowly and produced hemolysis on Bordet-Gengou medium; it did not reduce nitrates, ferment sugars, or produce indol. A 2 billion saline suspension of a 48 hour culture was agglutinated by pertussis-agglutinating serum (Lilly) through dilutions of 1:5120. Three mice inoculated intraperitoneally with 0.5 cc. of a saline suspension of 2 mg. of wet organisms from a 72 hour culture died within 24 hours. One tenth cc. of this suspension injected intradermally into a rabbit produced central necrosis surrounded by a purplish red indurated area that persisted for 10 days.

For the first experimental series a loopful of a 20 hour culture was transferred to the chorio-allantoic membrane of 6 11 day chick embryos, and from this the organisms were carried through 20 consecutive generations on the chick membranes. Embryos were taken for microscopic study from the first 3 and the 20th generations.

The bacilli from the 19th generation eggs grew more rapidly on Bordet-Gengou medium and agglutinated through only 1:160

serum dilution. Only 1 of 3 inoculated mice died within 24 hours. The remaining 2 died within 48 hours. In other respects the characteristics of the organisms as originally determined were unchanged. A control culture of bacilli transferred through 20 generations on Bordet-Gengou medium was agglutinated through a 1:1280 serum dilution.

Membrane and heart's blood cultures were not taken on the embryos sacrificed in the first 3 generations. In the 20th generation the 4th and 5th day postinoculation specimens showed 1 colony each from the blood culture; the 6th day specimen gave many colonies. The cultures of the membranes were positive through the 7 days of survival.

### THE CHORIO-ALLANTOIC LESION

In the gross the chorio-allantoic membranes 24 hours after infection usually showed a moderate edema, considerable hemorrhage and purulent exudate (Fig. 1). The appearance did not change greatly until the membrane began to dry on the 4th or 5th day postinoculation. The older membranes were considerably thickened when removed.

Smears made from the membranal exudate showed bacilli, red blood cells, polymorphonuclear leukocytes and monocytes. The bacilli were well preserved, small, and only rarely showed pleomorphism with chain formation. A few enlarged bacilli were scattered in the polymorphonuclear leukocytes and less frequently in the monocytes. Rarely a well stained mass of small bacilli was found in these cells. Eosinophilic polymorphonuclear cells predominated but the monocytes tended to increase after 48 hours. Degenerated cells were rarely found.

The microscopic examination of several membranes revealed a striking picture: 24 hours after inoculation (Fig. 2) areas of necrosis were seen in the hyperplastic ectodermal epithelium and in the adjacent vascular tissue, and fragmented nuclei were numerous and conspicuous. Throughout the edematous membrane were scattered numerous eosinophilic polymorphonuclear leukocytes and small foci of these leukocytes were frequent in the mesoderm and necrotic epithelium. In some areas the mesodermal cells showed a marked increase in size and intensity of staining, and a slight increase in number; this was especially notable about the blood ves-

sels where a deeply staining fibrous tissue resulted. At times areas of this thickened vascular wall were necrotic and infiltrated with polymorphonuclear leukocytes. There were large areas of hemorrhage containing masses of necrotic cells and the endothelium of the involved capillaries was degenerated. The endoderm was hyperplastic. No bacteria could be identified among the numerous nuclear fragments. Apparently this process had progressed until, within 48 hours after inoculation, hyperplasia of the mesodermal cells in an edematous tissue had produced a picture suggesting dense alveolar tissue, in the alveoli of which were numerous polymorphonuclear leukocytes. In the 72 hour membrane the tissue was more compact. The interstices between the deeply staining bands of hyperplastic fibrous tissue contained an eosinophilic fluid, a few well preserved leukocytes and fragments of degenerated cells. Necrosis of the tissue was not as prominent as in the earlier stages. The membrane from the 20th generation embryo 6 days postinoculation showed a deeply ulcerated area extending into the mesoderm and filled with masses of bacilli (Fig. 3). The 72 hour 3rd generation, and the 24 hour and 48 hour 20th generation membranes did not show the above described lesions, but appearances similar to *H. influenzae* infection. After 72 hours the membranes showed edema, fibrous tissue and large foci of intense blue staining mononuclear cells with prominent nucleoli and occasional foci of polymorphonuclear leukocytes. These membranes also were not markedly different from those of *H. influenzae*.

Thirteen embryos were taken for microscopic study, 6 from the earlier generations, representing infections of from 1 to 7 days, and 7, representing infections through 7 days, from the 20th generation of the organisms on eggs.

In the 24 hour, 48 hour, and 96 hour specimens from both groups there were scattered throughout the tissues small areas of hemorrhage in which necrotic red blood cells and an occasional thrombosed capillary were present. There was no associated cellular reaction or bacteria. Except for necrosis in the areas of hemorrhage, the endothelial cells appeared normal. In the older embryos there was the non-specific perivascular infiltration with polymorphonuclear leukocytes and monocytes similar to that found in *H. influenzae* infection.

## PULMONARY LESIONS

Two embryos of the foregoing series were remarkable. Numerous tiny bacilli were seen in the occasional ciliated epithelial cells of the esophagus (Fig. 12) in the 3rd generation embryo taken 5 days after inoculation of the membrane. There was no necrosis or cellular reaction of the underlying cells. No definitely ciliated epithelium could be found in the respiratory tract. An embryo of the 20th generation, 6 days after inoculation, showed a significant pathological picture in the lungs (Fig. 5). The cilia of the epithelial cells of the bronchi and air sacs enmeshed myriads of tiny deeply staining bacilli (Figs. 6, 7, 9, 10). In the midzonal and basilar portions of this ciliated epithelium were areas of granular necrosis containing many deeply basophilic fragments. At this stage only an occasional monocyte or leukocyte was seen infiltrating these areas. The muscularis appeared intact and a few monocytes and polymorphonuclear leukocytes were present in the peribronchial tissue. The bronchial lumen contained many polymorphonuclear leukocytes, monocytes, amorphous eosinophilic material, and occasionally a few extracellular and intracellular degenerated bacteria. In this embryo no well defined cilia were found in the pharynx or trachea, and in these situations the epithelial cells showed no necrosis. The non-ciliated bronchiolar epithelium was well preserved. Some of the bronchioles contained a dense occluding mass of leukocytes and degenerated bacteria. The alveolar epithelium, where preserved, was composed of large, pale ragged cells which in many areas were desquamated. The alveoli contained these desquamated cells, polymorphonuclear leukocytes, monocytes, and in several there were markedly degenerated bacteria. The interalveolar tissue was hyperplastic, the nuclei of the cells enlarged, and the fibrils thickened and deeply staining. There was an abundant interstitial infiltration of monocytes and polymorphonuclear leukocytes. In many situations where the alveolar epithelium was desquamated and the alveoli compressed by the thickened walls, the tissue appeared as an inseparable mass of fibroblasts, monocytes and polymorphonuclear leukocytes. A few areas of hemorrhage were present. Altogether the lesion constituted a diffuse bronchitis and bronchopneumonia involving the entire lung. The ciliated epithelium of the esophagus contained many bacteria but there was no evidence of necrosis.

In an attempt to reproduce this lesion in the lung, a 2nd series of 4 groups of embryos was inoculated. The 19th generation egg culture from the 1st series was used to initiate infection. It had been maintained through 4 transfers for 2 months on Bordet-Gengou medium.

Ten 13 day embryos were inoculated into one of the larger veins of the chorio-allantoic membrane with 0.05 to 0.1 cc. of a 1 billion per cc. saline suspension of a 36 hour culture. Two of these embryos were sacrificed at the end of 4 days and the 2 still living at 6 days were killed for study. Cultures taken from the mouth and heart's blood were negative and the microscopic study revealed nothing remarkable.

Ten day embryos were inoculated by forcing a needle directly into the body and introducing with a syringe 0.1 cc. of a 15 billion per cc. saline suspension of a 12 hour culture. Two of these survived to be autopsied 6 days later. The mouth cultures on both were positive and 1 had a positive blood culture. On microscopic examination no organisms or definitely ciliated cells could be found in the esophagus or respiratory tract.

The amniotic sac of 7 13 day embryos was inoculated with 0.1 cc. of a saline suspension of a 36 hour culture, 1 billion per cc. Two of these embryos were sacrificed at the end of 4 days. Microscopic examination showed nothing significant. One had definite ciliated cells but the mouth and blood cultures were negative. The other, which showed no ciliated epithelial cells, had a positive mouth culture and negative blood culture. Two embryos of this group survived to be sacrificed for study at 6 days. The mouth cultures on each showed numerous colonies; the blood cultures were negative. Microscopically each embryo had numerous well preserved bacteria on the ciliated epithelium of the pharynx, trachea, bronchi, air sacs and esophagus. One embryo showed no apparent local reaction to this infection. The 2nd embryo showed only very rarely an early granular necrosis of the epithelium and a slight infiltration of the submucosa with polymorphonuclear leukocytes. A few secondary bronchi, bronchioles and alveoli contained a moderate number of monocytes, polymorphonuclear leukocytes and degenerated bacteria. Some groups of terminal alveoli contained desquamated alveolar cells and occasional degenerated bacteria, polymorphonuclear leukocytes and monocytes.

In these areas the alveolar walls were poorly defined and the interstitial tissue showed a few monocytes and polymorphonuclear leukocytes; some interstitial cells were hyperplastic and others appeared compressed, having a distorted deeply staining nucleus.

A 4th group, consisting of 9 12 day embryos, was inoculated in the same manner as the 3rd group, using a 15 billion suspension of a 12 hour culture. Only 1 of these embryos was alive on the 4th day; it was then sacrificed. The mouth and heart cultures were positive. Microscopically the epithelial cells of the esophagus showed well defined cilia and only in these situations were organisms found.

These experiments thus far indicate that the method of choice for successful pulmonary infection is by way of the amniotic fluid by means of which the bacilli are brought, probably by respiratory movements, into direct contact with ciliated epithelium, which seems to be the best medium of the host for stimulating their growth. Occasional infection of the lungs, however, may occur from primary infection of the chorio-allantoic membrane.

## DISCUSSION

There is excellent clinical evidence of the experimental production of whooping cough in animals <sup>4-8</sup> and in man <sup>9</sup> by the introduction of *H. pertussis* into the respiratory tract. The pathological lesions have been investigated in only a few of these animals. Sauer and Hambrecht <sup>10</sup> autopsied two of their monkeys during the experimental whooping cough infection. They described bacilli in the cilia of respiratory epithelium, and peribronchial inflammation, but did not find the midzonal necrosis and leukocytic infiltration that we have found in cases showing peribronchitis to the extent described in their Monkey A 5. However, the monkeys were sacrificed and a terminal picture was not to be expected. Shibley <sup>9</sup> found no bacteria in the cilia of his autopsied chimpanzee.

That *H. pertussis* causes the bronchial infiltration in whooping cough has been taken for granted except when this infiltration has been attributed tentatively to the action of an undemonstrated virus. That the pneumonia is associated specifically with *H. pertussis* infection has been concluded by Smith,<sup>11</sup> Fonteyne <sup>12</sup> and others; and that *H. pertussis* can produce an interstitial pneu-

monia has been indicated by Sprunt, Martin and Williams.<sup>18</sup> However, it has never been unquestionably established that *H. pertussis* alone can produce the lesions found in man and experimental animals with whooping cough. This proof has been lacking largely because it is impossible to rule out other associated or secondary infections in man and animals.

The chick embryo offers an unparalleled opportunity to study the pathological process of a pure infection. The accessibility of the respiratory tract through the amniotic fluid aids greatly in the investigation of an infection by an organism to which the respiratory tissues are susceptible.

By this investigation it has been established that the chick embryo is susceptible to infection with *H. pertussis* and that the lesions found in fatal cases of human whooping cough can be produced in detail in the chick embryo lung by *H. pertussis* alone. These facts indicate that *H. pertussis* is the inciting cause of whooping cough in man, for in these experiments it cannot be assumed with reason that the pulmonary infection was initiated by an associated virus or by any other microorganism.

The experiments recorded show that *H. pertussis* tends to localize specifically on and in the ciliated border of respiratory and esophageal epithelium. There is no other tissue in this host, removed from the primary infection in the chorio-allantoic membrane, where evidences of metastatic infection have been found. Although the bacilli may rarely infect the lung from the primary membranal lesion, experiments thus far indicate that their admission to the lung through amniotic fluid is a preferable route.

There seems to exist a specific reproductive relation between the ciliated border of epithelium of the respiratory tract and the bacilli; and the epithelium of the bronchi and bronchioles appears to be more advantageous for the growth of this bacterium than that of the trachea and elsewhere.

The growth of the bacilli in the ciliated border is usually associated with an injury, leading to necrosis, of epithelial cells situated in the midzonal and basal layers of the bronchial and bronchiolar epithelium. This injury is responded to by an exudate of fluid, polymorphonuclear and mononuclear leukocytes within the epithelial layer, in the lumens, and in peribronchial and interstitial tissues of the lung in general. The pulmonary alveoli may be filled



with cellular exudate, desquamated cells, fluid and degenerating bacteria. There is as yet no definite evidence that the bacilli grow in the lung except in association with the ciliated border of epithelium. From these sites it is probable they are borne by respiratory movements to the alveoli where they degenerate, but they may induce here also degeneration and necrosis of non-ciliated respiratory epithelium.

In the chick embryo, therefore, the lesions indicate that the growing *H. pertussis* liberate an injurious substance which acts primarily on non-ciliated respiratory epithelium, causing degeneration and necrosis; and *H. pertussis* in this bird likewise can cause bronchopneumonia, in addition to the inflammatory reaction in and about the larger air channels.

In sections of widely expanded air sacs connected with bronchi it is possible to find isolated islands of ciliated epithelium containing abundant growth of bacilli, while the surrounding non-ciliated epithelium contains none (Fig. 11). Under these circumstances it is only beneath the ciliated infected cells that necrosis of epithelium occurs. This indicates quite clearly that the injurious effect is the result of some toxic product of the growing bacilli acting locally.

Rather generalized perivascular infiltration in the embryo may be interpreted as an indication of a more widespread effect of such an injurious substance or substances.

This is the report of preliminary work. Further investigations along many lines are suggested. By this experimental approach it may be possible to determine the relation of the factors that establish the infection and to study the progress of the lesions.

The importance of the ready access of the microorganism to the susceptible tissue is indicated by the experimental record. That a particular type of cell may be of significance in the pathogenesis of an infection has been indicated elsewhere,<sup>14</sup> and the suggestion has been made that there might be a reproductive relation between *H. pertussis* and the ciliated respiratory epithelium.<sup>15</sup> That such a relation exists is strongly indicated in these studies. Although it is difficult to be certain of the absence of ciliated epithelium in cells with a cuticular margin overlaid with eosinophilic fluid and amorphous material, in a large series of embryos definitely ciliated respiratory epithelium was not found before the 15th day and

usually not until the 17th day. The few ciliated esophageal cells usually have well defined cilia by the 14th or 15th day.

Possibly the variation in the picture produced by the infection of the 2 embryos of the same age given equivalent amounts of the same suspensions is due to a variation in the time of development of the ciliated epithelium, or to the relative position of the embryo in the amniotic cavity, or to the establishment of or a variation in the respiratory movements. This latter possibility is suggested by the work of Snyder and Rosenfeld<sup>16</sup> on fetal respirations.

Phenomena involved in the specific localization of bacteria in the infected host are susceptible of investigation by the method used.

### SUMMARY AND CONCLUSIONS

Chick embryos are susceptible to infection with *H. pertussis*. Infection of the respiratory tract, including the lungs of the embryo, has been induced by infection of the chorio-allantoic membrane and by inoculation of the amniotic fluid with *H. pertussis*.

The pulmonary lesions of human whooping cough are reproduced in detail by the pulmonary infection of the embryo. These are characterized by growth of *H. pertussis* on the ciliated border of respiratory (and other) ciliated epithelia, necrosis and inflammation of the middle and basal layers of the epithelial membrane, intrabronchial and peribronchial cellular exudation, and alveolar and interstitial pneumonia.

These experiments indicate that *H. pertussis* alone is the inciting cause of whooping cough.

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## DESCRIPTION OF PLATES

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### PLATE 138

- FIG. 1. Lesion on chorio-allantoic membrane 48 hours after inoculation with *H. pertussis*.
- FIG. 2. Section of chorio-allantoic membrane 24 hours after inoculation. Edema and polymorphonuclear reaction especially evident.  $\times 650$ .
- FIG. 3. Lesion on membrane showing necrosis. Rarely so destructive.  $\times 55$ .
- FIG. 4. Lung of normal embryo to be compared with Fig. 5.  $\times 30$ .



FIG. 1

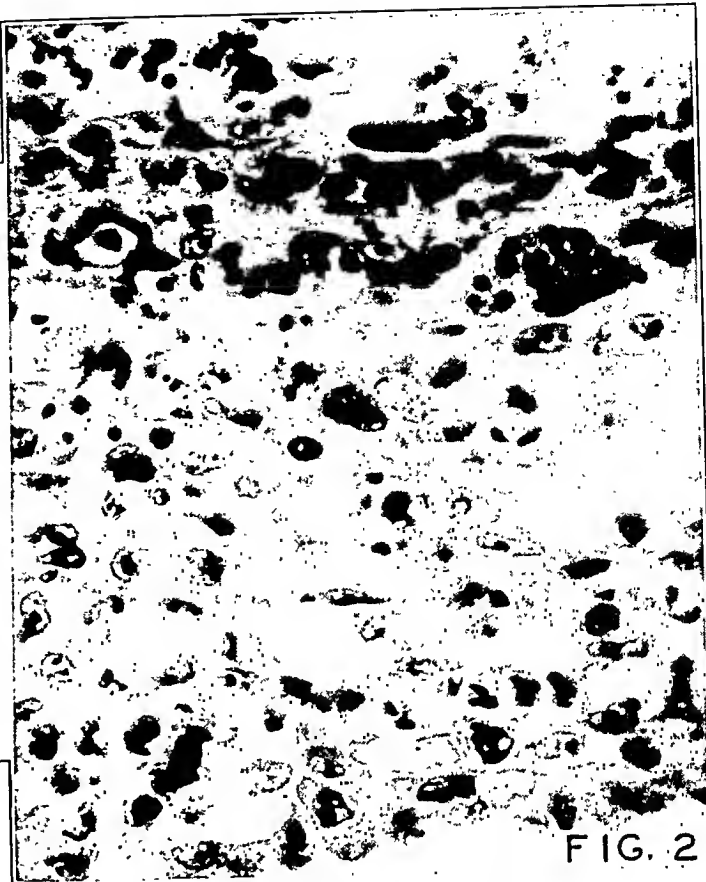


FIG. 2

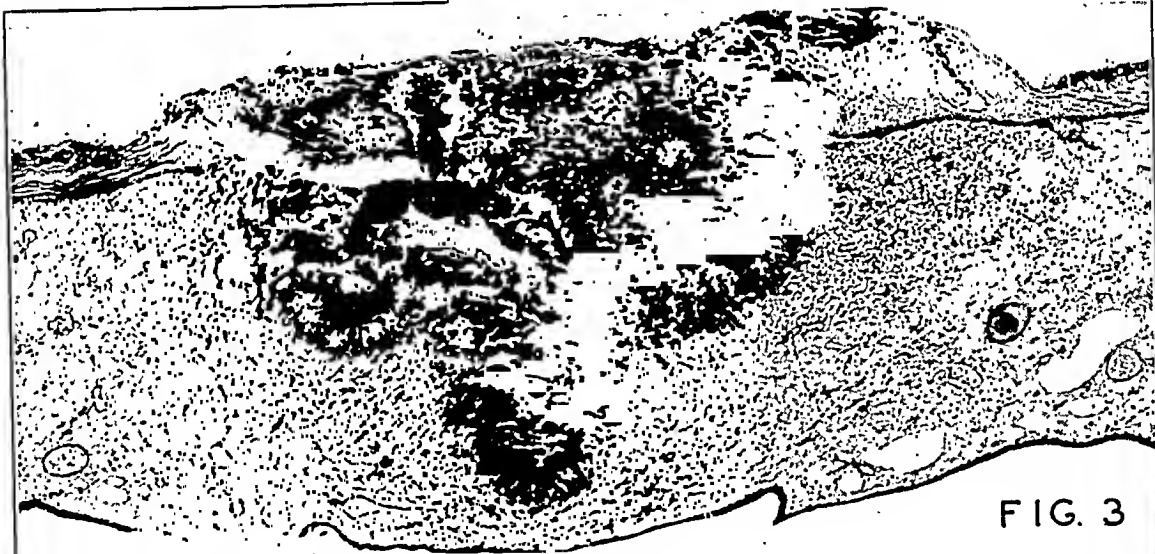


FIG. 3

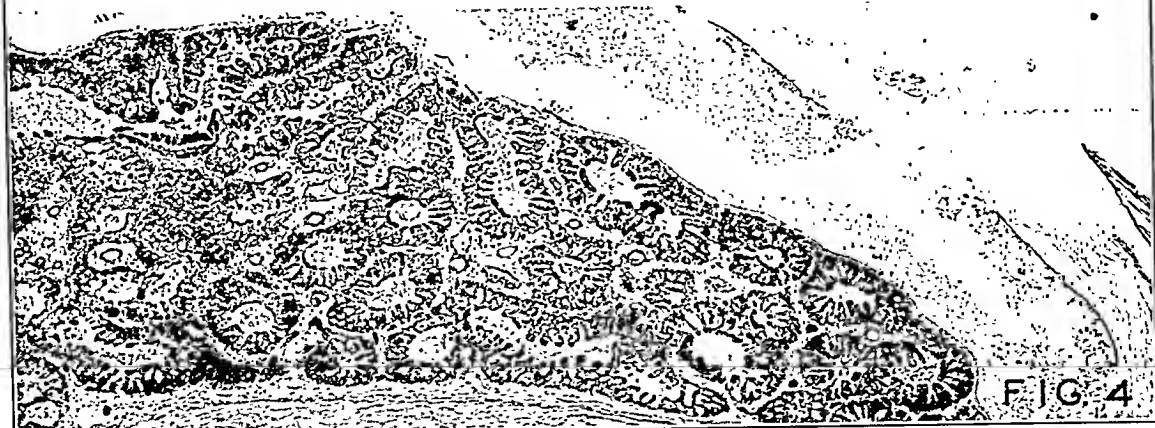


FIG. 4

PLATE 139

- FIG. 5. Lung of embryo 6 days after inoculation of membrane with *H. pertussis*. Note intrabronchial exudate and interstitial and bronchopneumonia. Compare with Fig. 4.  $\times 30$ .
- FIG. 6. Higher power of bronchus in upper third of Fig. 5. Note exudate in lumen, necrosis and infiltration of epithelium, peribronchial and interstitial inflammation, and bronchopneumonia.  $\times 190$ .

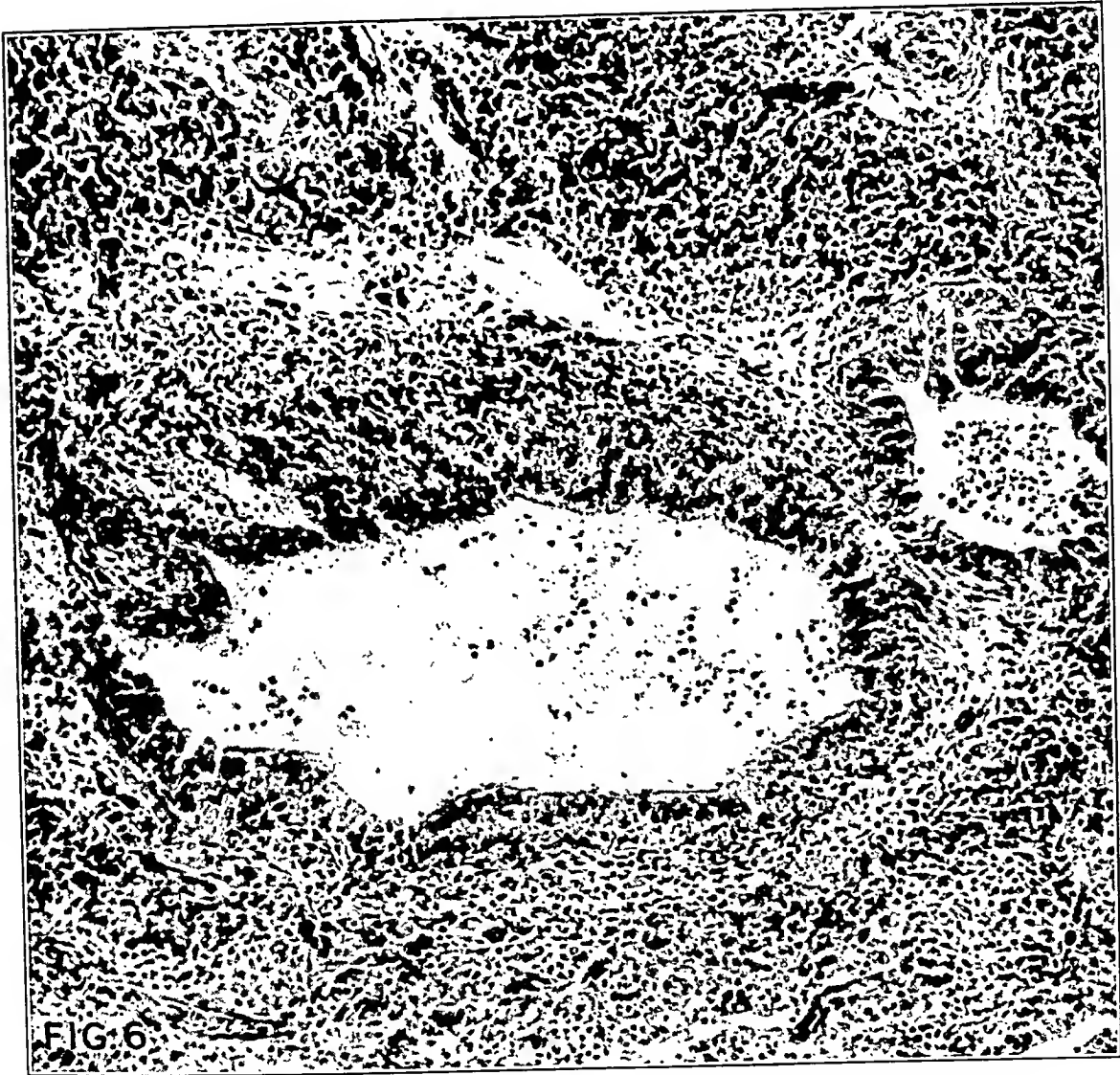
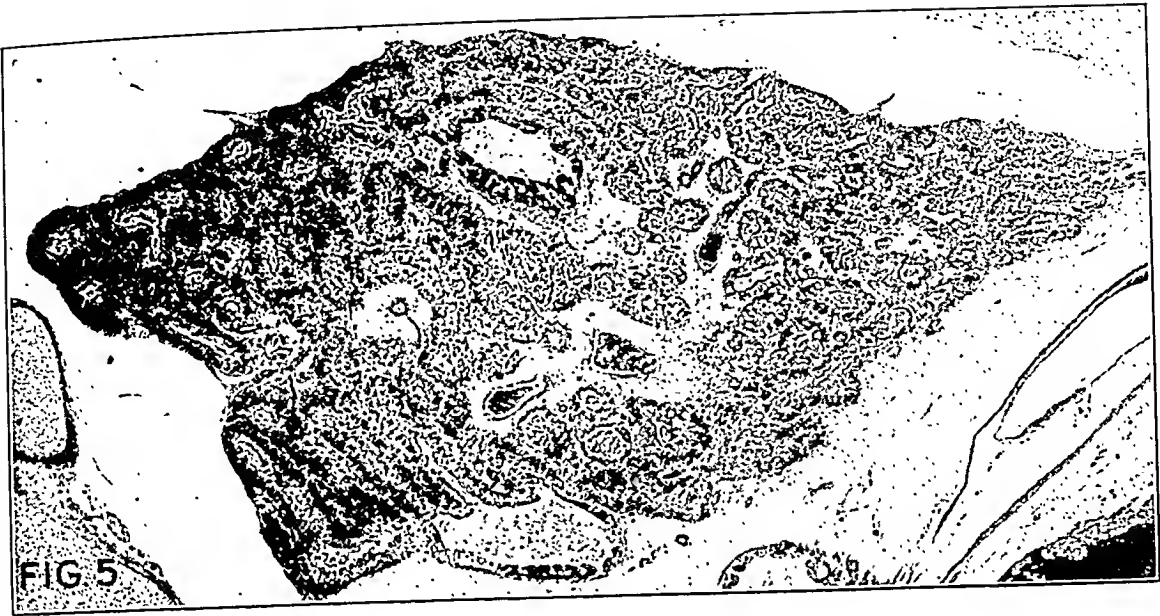


PLATE 140

- FIG. 7. Small bronchus in embryonic lung showing necrosis, desquamation and inflammation in and beneath epithelium.  $\times 400$ .
- FIG. 8. Small bronchus from a case of human pertussis for comparison with Fig. 7. Note similar injury and inflammation.  $\times 120$ .
- FIG. 9. Higher magnification of embryo bronchus showing zone of bacilli in ciliated border, and necrosis and infiltration of middle and basal layers of epithelium. Exudate in lumen.  $\times 625$ .

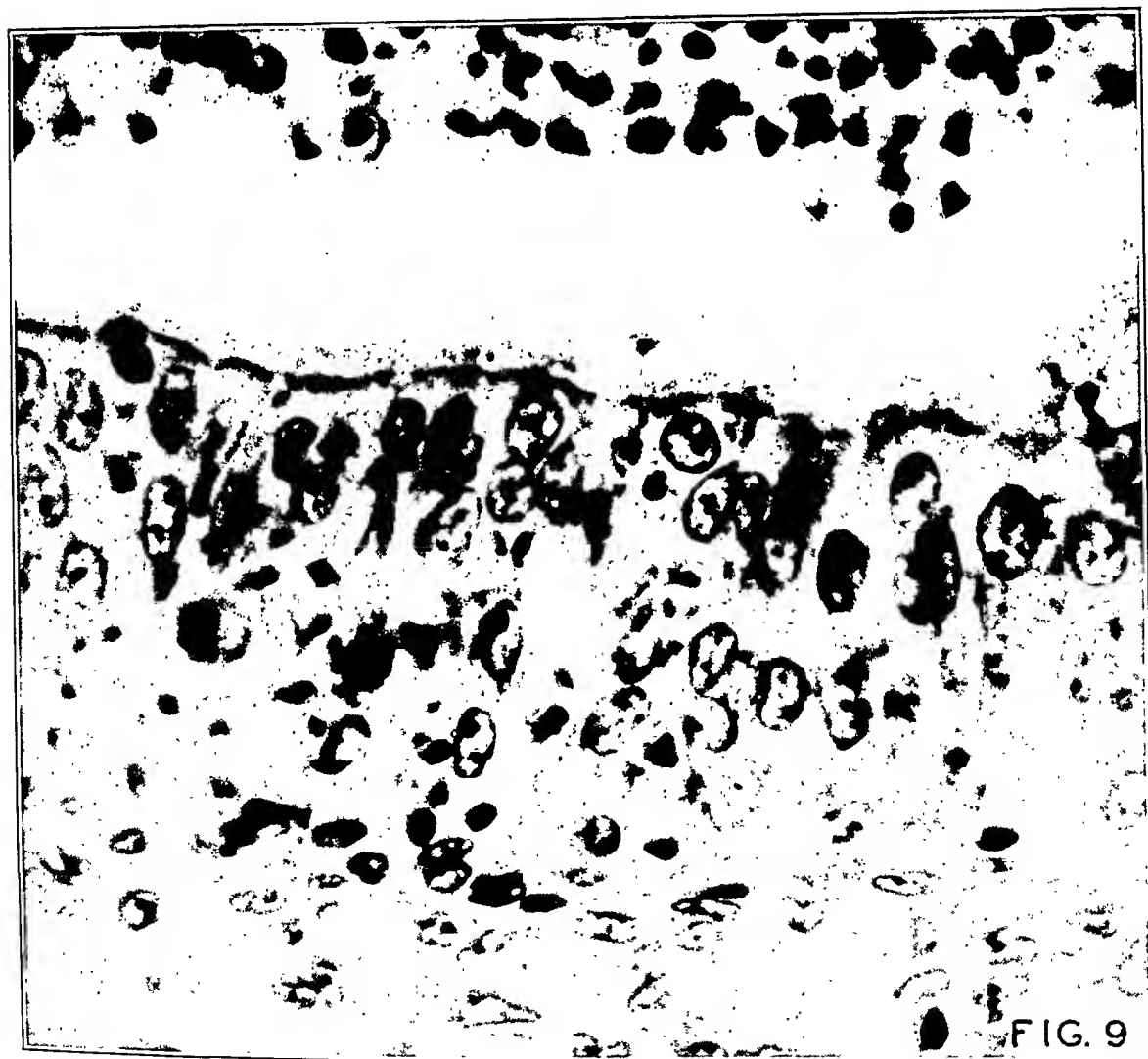


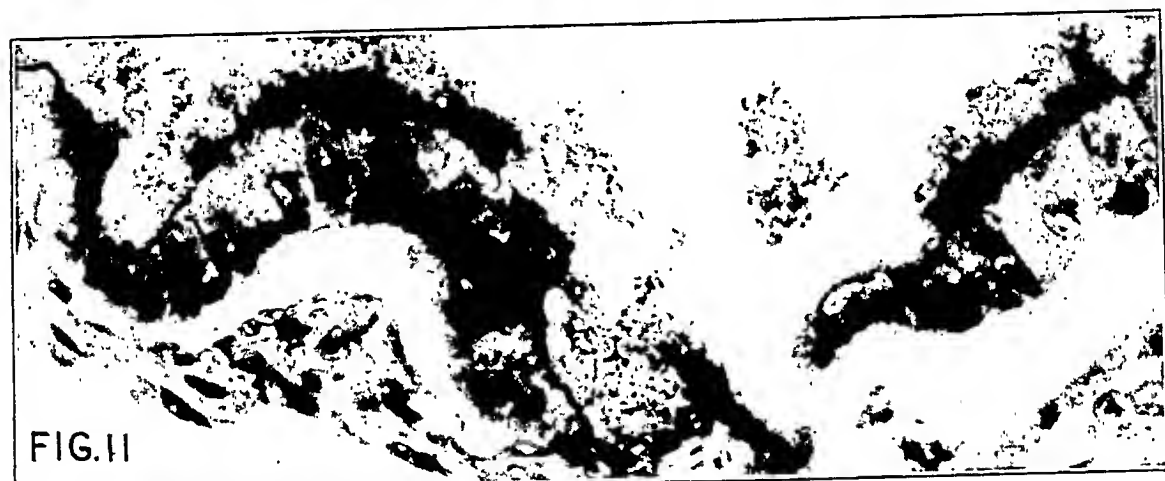


PLATE 141

FIG. 10. Bronchial epithelium of chick embryo showing bacilli among cilia and degeneration and necrosis of midzonal epithelium.  $\times 2300$ .

FIG. 11. Islands of ciliated epithelium covered with masses of bacilli in expanded air sac from bronchus of chick embryo lung.  $\times 700$ .

FIG. 12. Isolated ciliated epithelial cells holding clumps of bacilli in esophagus of embryo chick.  $\times 700$ .





# SPECIFICITY OF THE LESION OF EXPERIMENTAL MUMPS \*

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The object of this study was to find out whether the lesions produced in the parotid glands of *Macacus rhesus* monkeys by injecting mumps virus into Stenson's duct are microscopically specific, as stated by Johnson and Goodpasture, or non-specific and reproducible by the injection of various other substances, as Levaditi and coworkers contend.

Johnson and Goodpasture,<sup>1</sup> after reviewing the brief literature on the histopathology of human mumps and describing the microscopic appearance of glands taken at the height of the experimental disease in monkeys, conclude that the essential lesion produced by the mumps virus is focal degeneration and necrosis of the acinar epithelial cells, while the inflammatory cellular response is secondary thereto. They also describe pink staining, round, vacuolated inclusion bodies in the cytoplasm of degenerated and necrobiotic cells, and hazard the suggestion that these are specific in the mumps infection.

Levaditi and coworkers<sup>2</sup> agree that mumps is caused by a filtrable, glycerin-resistant pathogen, and can be transmitted to monkeys, but they concluded, on inoculating with several control substances (normal saliva, yeast, white of egg, horse serum, tapioca, herpes virus, and lymphogranuloma virus), that there was only a quantitative difference between the lesions produced and those caused by mumps. All types of inoculums produced a simple interstitial mononuclear inflammation without much epithelial involvement, and only by counting the number of lymphocytic foci in a microscopic field could one determine which glands were infected with mumps. Levaditi *et al.*, do not mention any foci of acinar degeneration and necrosis.

Since Johnson and Goodpasture used as controls only monkeys inoculated with normal monkey parotid suspensions in saline, it has seemed important to repeat their experiments, both with mumps virus and with several other control inoculums.

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## MATERIALS AND METHODS

The parotid glands of 37 *Macacus rhesus* monkeys have been studied. Inoculations were made by syringe and cannula into the parotid ducts of etherized animals. In those inoculated with mumps virus the onset of mumps was detected by secondary enlargement of the glands to palpation and by pitting edema of the jowl, occurring generally about the 6th to 8th day after inoculation and accompanied often by a secondary rise of temperature. When these signs appeared the monkeys were etherized and exsanguinated and the parotid glands were fixed in a modified form of Schaudinn's fluid,\* to be sectioned and stained by hematoxylin and eosin. No special stains were employed. In monkeys inoculated with control materials there was no secondary development of clinical mumps, but the animals were killed after intervals similar to those having mumps. The following inoculums were used:

1. Centrifuged saline suspension (20 per cent by weight) of the crushed parotid glands of monkeys at the peak of infection with experimental mumps. Two strains of virus were used: one, that of Johnson and Goodpasture, up to its 35th monkey passage; the other one recently derived from human saliva.

2. Centrifuged saline suspension of parotid glands infected with mumps boiled for 10 minutes.

3. Centrifuged saline suspensions (20 per cent) of the crushed parotid glands of normal monkeys.

4. Centrifuged saliva from children during the first 2 days of clinical mumps.

5. Berkefeld V filtrate of normal monkey saliva.

6. Berkefeld V filtrate of saliva from monkeys recently recovered from mumps.

7. A 5 per cent solution of granular mucin in saline.

8. A 7.5 per cent solution of soluble starch in saline.

9. Physiological saline solution.

10. Fresh white of egg.

11. Horse serum.

12. A 1:400 suspension of vaccinia virus from the chorio-allantoic membranes of chick embryos.

\* Saturated solution of mercuric chloride, 2 parts; 60 per cent alcohol, 1 part.

The size of the inoculum varied from 2 to 4 cc. in each duct.

All the above preparations were cultured and, with a few exceptions noted later, were found sterile at the time of injection.

### HISTOLOGICAL EXAMINATION

A number of the sections studied came from monkeys that had been inoculated with mixtures of virus tissue and saliva or serum, used in immunity and neutralization experiments, and which had died from 1 to 7 months after inoculation from tuberculosis, Flexner's dysentery, or from injuries by other monkeys. Their glands showed the late and ultimate effects of the mumps infection. No attempt was made, however, to study the entire pathogenesis of the infection from day to day with biopsies or serial killings. Nor was it considered practical to count the number of inflammatory foci, as was done by Levaditi and coworkers, since nearly every gland studied showed the greatest irregularity in distribution of lesions.

Thirty-seven parotid glands were studied which had been removed 6 to 8 days after injection with infectious human saliva or infectious monkey parotid by way of Stenson's duct. All showed essentially the same changes, of which the earliest was degeneration and necrosis of the epithelial cells in scattered acini, generally affecting first those at the center of the lobule, and later those farther out. Following focal necrosis the involved acini were invaded by monocytes and the entire periductal area, as well as the interacinar tissue in the vicinity, became infiltrated with lymphocytes and monocytes, occasional plasma cells, and rare polymorphonuclear neutrophils and eosinophils. There were edema and fibrin deposition in the intralobular and interlobular connective tissue of the inflamed areas, and occasional small recent hemorrhages. In any one section from a gland at the active stage of the infection all these changes could usually be seen, different lobules and different portions of one lobule showing acini with hydropic cells, or swollen or pyknotic nuclei, and also acini with necrotic debris which was being ingested by phagocytes to be replaced by edema and lymphocytic exudate. Neutrophilic leukocytes were absent, except in the ducts of one of the glands inoculated with unfiltered (contaminated) human saliva.

Cytoplasmic inclusion bodies of the type described by Johnson

and Goodpasture were found in 19 of the 37 mumps-inoculated glands. These bodies are pink staining, round or oval, 3 to 10  $\mu$  in diameter, often contain small vacuoles, and are usually surrounded by a narrow clear zone in the cytoplasm. They were discovered more frequently in the epithelial cells of acini showing degenerative changes, or adjacent to involved areas of the gland.

One gland removed 11 days after inoculation with mumps showed the same picture as that described above, *i.e.* necrosis, lymphocytes, inclusion bodies, edema and hemorrhage. The period of invasion in this case was longer and swelling did not appear until the 10th day.

Eighteen glands were examined from monkeys that had died or had been killed weeks or months after the initial mumps infection. In these glands the only positive finding was an occasional, usually small collection of lymphocytes in the periductal connective tissue, not involving the interacinar tissue. There was no fibrosis, necrosis, hemorrhage or edema, nor were inclusion bodies found.

One gland was studied which had been injected 6 days before with boiled mumps-infected gland suspension. It showed no pathological changes, with the exception of an occasional group of periductal lymphocytes.

One gland was removed at autopsy 11 days after injection with a suspension of normal monkey parotid gland. It showed a minimal number of periductal lymphocytes. An adjoining lymph node contained more monocytes in the medulla than are seen normally.

In one gland, removed 6 days after injection with the Berkefeld filtrate of saliva from a normal monkey, there was no change except the presence of a few periductal lymphocytes. The same was true of the other gland of the same animal, injected with a filtrate of saliva from a monkey that had had mumps a few weeks before.

One gland was examined 7 days after the injection of a 5 per cent solution of granular mucin in saline. It could be distinguished from a normal gland only by the presence of a few more periductal lymphocytes than are seen normally, and a few eosinophiles.

One gland received a 7.5 per cent solution of soluble starch in saline, and 7 days later showed only a slight increase of periductal lymphocytes, together with a change not seen in any other gland of this series. In several lobules of the section there were irregular areas in which the acini, though not necrotic, exhibited a disarrangement of the cells, which took a much lighter, pinker stain

than is seen normally. The appearance was somewhat similar to that of an early stage of Minkowski's degeneration of the pancreas.

One gland that had received physiological saline solution 6 days previously showed no pathological change whatever. Several normal (uninjected) glands were examined, and it was found that the presence of a few lymphocytes, or even of a small collection in the vicinity of the ducts should not be regarded as pathological.

One gland was injected with fresh white of egg. At autopsy 6 days later it appeared grossly normal and on section showed only a minimal amount of edema and periductal lymphocytic infiltration.

One gland, injected with horse serum 6 days before, showed slight edema, small hemorrhages, an increase in the periductal lymphocytes, and a few eosinophiles, but no other changes.

One section from a gland injected 6 days before with normal saliva from a man who had had mumps years before showed an increase of lymphocytes, both periductal and interacinar, an occasional polymorphonuclear leukocyte in the periductal tissues, and numerous cytoplasmic inclusions. There was no focal acinar necrosis of the type seen in mumps but an occasional cell or two, here and there, was necrotic. The inoculum in this case, being a centrifuged supernatant fluid and not a filtrate, was contaminated by bacteria.

One gland was examined on the 7th day after receiving 1 cc. of a dilution of vaccinia virus. It showed a large number of lymphocytes, both interacinar and periductal, marked edema, hemorrhage, and a small number of neutrophilic leukocytes, but no necrosis or inclusions. The clinical course was much more rapid and the gland was firmer to the touch than in mumps infection.

One monkey, inoculated in one parotid with mucin and in the other with saline solution, developed a suppurative infection of the mucin-injected parotid. The inoculum was cultured and found to contain *Staphylococcus aureus*. Sections of the glands showed multiple abscesses of the infected gland, with some fibroblastic reaction and many plasma cells. Edema, hemorrhage, and lymphocytic infiltration were also abundant, and there were large and small areas of necrosis, some of which could not be distinguished from those caused by mumps.

The saline-injected gland showed no suppuration, but edema, heavy lymphocytic infiltration, acinar necrosis and inclusion



bodies were found. This lesion was indistinguishable from that of mumps and an adequate explanation of it is not at hand as it was not reinoculated into the monkeys to rule out mumps. However, there was no gross edema of the gland, which was of normal size.

Two other glands examined showed staphylococcal infections: one, which also showed mumps, from a contaminated inoculum; the other, 7 months after inoculation, from a traumatic infection. Both showed suppurative foci with polymorphonuclear leukocytes in marked predominance.

### CONCLUSIONS

From the above observations it is concluded that:

1. Focal acinar necrosis, *i.e.* necrosis involving all or most of the cells of an acinus, is the fundamental lesion of experimental mumps, the lymphocytic reaction, edema and hemorrhage being subsequent factors.

2. None of the inert or infectious substances injected give this particular type of necrosis, though some of them may cause the death of a cell here and there or produce an abscess destroying large numbers of acini.

3. Cytoplasmic inclusion bodies, though found in over half the cases of mumps, may also be found in glands injected with normal saliva, and cannot therefore be regarded as a specific sign of mumps infection.

4. The abundance and interacinar location of the lymphocytic exudate can be suggestive evidence that the lesion is due to infection rather than to an inert substance, but do not aid in distinguishing between different types of infection.

It should be stated that in no instance following injections of the parotid with material other than that of mumps did a secondary enlargement, with edema of the gland associated with elevation of temperature, occur. This seems to be characteristic of mumps infection, as is likewise the histopathological lesion.

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# CHANGES IN THE TEETH FOLLOWING PARATHYROIDECTOMY\*

## I. THE EFFECTS OF DIFFERENT PERIODS OF SURVIVAL, FASTING, AND REPEATED PREGNANCIES AND LACTATIONS ON THE INCISOR OF THE RAT

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### INTRODUCTION

Since the incisor teeth of the albino rat reflect with a high degree of accuracy and sensitivity the fluctuations in calcium and phosphorus metabolism, they have proved to be a valuable indicator in studying the physiology of the parathyroid glands.

The purpose of this investigation was, therefore, to study the response of the incisor of the rat to the following experimental conditions: (1) short survival following parathyroidectomy (3–20 days); (2) long survival following parathyroidectomy (40 days or more); (3) periodic fasting superposed on parathyroidectomy of long survival; and (4) repeated pregnancies and lactations superposed on parathyroidectomy of long survival.

### REVIEW OF LITERATURE

Erdheim<sup>1</sup> was the first to study the dental changes in the incisors of parathyroidectomized rats. He reported the following changes. The enamel surface showed opaque spots and the teeth fractured 6 to 10 weeks after the operation. The dentin showed incomplete or no calcification, a wide predentin, and vascular inclusions. The enamel epithelium was atrophied and folded. Atypical enamel formation occurred within the enamel epithelium.

Toyofuku<sup>2</sup> made a more detailed histological analysis of the incisors of 27 rats that survived 1–135 days after parathyroidectomy. He confirmed Erdheim's findings and added observations made on roentgenograms. The findings of Erdheim and Toyofuku have been repeatedly confirmed and supported by a number of

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investigators (Preiswerk-Maggi,<sup>3</sup> von Spreter<sup>4</sup>). In the above investigations diet was an important determinant but unfortunately the exact diets used were not recorded.

In 1911 Erdheim<sup>5</sup> removed the parathyroids and transplanted them in the abdominal wall of the same animal. He thus produced a temporary state of parathyroidectomy which was registered in the form of an imperfect calcification of the dentin that was laid

TABLE I

*Data on 18 Parathyroidectomized Animals of Group I Arranged According to Postoperative Survival*

Rat. No.	Age at operation	Weight at operation	Postoperative survival
	<i>days</i>	<i>gm.</i>	<i>days</i>
110	46	84	3
82	21	35	4
88	79	138	5
36	23	47	6
50	24	30	7
55	24	31	8
9	32	52	10
53	24	28	14
54	24	30	13
43	21	37	13
44	21	37	14
52	24	27	14
51	24	25	15
41	21	35	15
42	21	38	15
49	23	31	16
37	23	53	20
38	23	53	20

down during the time that elapsed between the removal of the parathyroids and the "taking" of the transplant. This zone Erdheim called the transplantation stripe. In 1914, in his classic work "Rickets and Parathyroids," Erdheim<sup>6</sup> pointed out that changes in the incisors of rachitic rats were the same as those in parathyroprivic rats.

#### MATERIAL AND METHODS

This report is based on 100 parathyroidectomized albino rats. In the great majority the age at the time of operation ranged from 21 to 79 days (Table I). The range of postoperative survival was from 3 to 159 days (Tables I to III).

TABLE II

*Data on 57 Parathyroidectomized Animals of Group II Arranged According to Postoperative Survival*

Rat No.	Age at operation	Weight at operation	Postoperative survival *
	<i>days</i>	<i>gm.</i>	<i>days</i>
67	55	106	39
68	55		39
25A	27	57	42
27A	27	47	43
14	117	158	43
45	21	36	47
47	21	37	47
48	21	34	47
6B			48
7B			48
9B			48
10B			48
3B			49
5B			49
61	111	152	52
6A	40	103	56
10A	41	76	56
13A	41	68	56
14A	41	68	56
4A	39	84	57
5A	39	70	57
17A	42	69	57
1A	38	62	58
3A	38	54	58
1	32	74	66
2	32	76	66
3	32	86	66
4	31	74	66
7	32	77	66
31	39		82
901	34		83
903	34		83
904	34		83
905	34		83
906	34		83
907	34		83
29	23	49	98
30	23		98
32	23	49	98
113	47	110	120
116	47	123	120
119	23	32	125
120	23	33	125

\* These animals were treated with calciferol or parathyroid extract several days before sacrificing. The indicated postoperative survival refers to the survival up to the time of treatment.

TABLE II (Continued)

Rat No.	Age at operation	Weight at operation	Postoperative survival *
	<i>days</i>	<i>gm.</i>	<i>days</i>
123	23	34	130
125	23	34	133
126	23	29	133
108	46	99	135
111	46	83	135
112	46	97	135
100	41	92	140
101	41	107	140
96	46	103	157
64	93	146	157
66	55	108	157
91	82	192	159
92	82	168	159
93	82	169	159

\* These animals were treated with calciferol or parathyroid extract several days before sacrificing. The indicated postoperative survival refers to the survival up to the time of treatment.

TABLE III

*Data on 14 Parathyroidectomized Animals of Group III Subjected to Fasting Every 7th Day*

Rat No.	Age at operation	Weight at operation	Postoperative survival
	<i>days</i>	<i>gm.</i>	<i>days</i>
H 40	63	137	105
H 42	63	122	105
H 44	65	110	105
H 50	67	147	105
H 52	67	159	105
H 53	67	162	105
H 55	67	185	105
H 56	67	160	105
H 70			105
H 80	70	87	105
H 86	70	69	105
H 87	71	117	105
H 90	72	95	105
H 92	72	101	105

On the basis of the experimental history, these animals may be divided into 4 groups:

Group I consists of 18 animals of short postoperative survival (3-20 days, Table I).

Group II consists of 57 animals of longer postoperative survival (39-159 days). These animals were treated with calciferol or parathyroid extract several days before death (Table II).<sup>\*</sup> They were included in this study because the histological findings in that portion of the incisor formed prior to treatment were fully representative of the changes produced by parathyroidectomy alone. The histological effects of the treatment on parathyroprivic dentin which was found to be confined to the portion that was formed and calcified during and subsequent to the time of treatment are described in the subsequent report.<sup>7</sup>

Group III consists of 14 animals of a postoperative survival of 105 days. During this period the rats were subjected to fasting on every 7th day (Table III).

Group IV consists of 11 rats of long postoperative survival (4 months to 1 year) which were subjected to repeated pregnancies and lactations. These animals are part of the series described in a previous report by one of us (Chandler<sup>8</sup>).

Similar histological studies were carried out on 27 litter-mate controls, of which 6 were subjected to unilateral parathyroidectomy; 6 were subjected to a sham operation in which the thyroids and parathyroids were exposed; and 9 were treated with parathyroid extract or calciferol. In addition, the histological data of a number of controls from the same colony and within the same age limits as the experimental animals were available from a previous study (Schour, Tweedy and McJunkin<sup>9</sup>).

The controls showed a normal histological picture in the dental tissues. The 9 animals that were treated with parathyroid extract or calciferol showed a normal picture in the portion of the incisors that was formed and calcified previous to the treatment.

The diet consisted of Purina Fox Chow *ad libitum* which has been found to be adequate in calcium, phosphorus, proteins, carbohydrates and fat. In addition the animals were given cheese and

<sup>\*</sup> We are indebted to Mead Johnson and Company for the calciferol used in these experiments. A portion of the parathyroid extract was supplied through the courtesy of Eli Lilly and Company.

cabbage twice and beef once a week. The animals belonged to the colony of the Loyola University Medical School and were originally derived from the Wistar strain.

Parathyroidectomy was performed by one of us (S. B. C.). Serial sections were prepared of all tissue removed and all incompletely parathyroidectomized animals were discarded. While the incidence of accessory parathyroid tissue was not determined by histological examination of serial sections of the entire neck regions from all animals used, similar studies recently carried out on 68 animals from the same colony revealed 5 animals with accessory tissue. We therefore regard the rats as completely parathyroidectomized since the amount of accessory tissue observed in the animals of this colony is quite small in every instance studied and the number of animals possessing residual parathyroid is less than 10 per cent.

After parathyroidectomy the rate of eruption of the incisors was measured. The heads of the animals were fixed in 5 per cent formalin immediately after death. They were then cut mid-sagittally and X-rayed. The tissues were washed, decalcified in 5 per cent nitric acid, dehydrated, and embedded in celloidin. Sections were cut in serial order and stained with hematoxylin and eosin. The majority were longitudinal sagittal sections of the upper and lower incisors. A small number of ground sections were prepared.

## GROSS FINDINGS

### *Findings in the Living Animals*

*Gross Changes:* The gross changes were similar to those repeatedly reported in the literature but appeared to occur later in respect to survival time. No gross changes were observed in Group I. In Group II the exposed enamel surface occasionally showed unpigmented spots and presented an opaque appearance. These changes were found to be more consistent in Group III. The defective pigmentation and opaque appearance of the incisors were more marked in Group IV. In most instances the teeth were elongated.

The exposed portion of the enamel frequently showed a fine but distinct ring-like stratification which could be recognized with the naked eye.

*Measurements of Rate of Eruption:* The weekly rate of eruption of the upper and lower incisors in 10 parathyroidectomized animals was determined for an average period of 10 weeks. These rats were obtained from Group II and were measured previous to their treatment with parathyroid extract or calciferol. The results indicate an average normal weekly rate of 2 mm. in the upper and 2.8 mm. in the lower incisors. Similar measurements on normal animals were in the same range.

### *Radiographical Findings*

The roentgenogram of the incisor of the normal mature rat presents a curved and partially hollow cylinder with a sharp incisal edge. The shadow of the tooth has a smooth and sharply delineated external outline and is dense in its distal third or half. Beginning with the middle third, because of the increasingly large radiolucent pulp, this shadow splits into a tapering convex (labial) and a tapering concave (lingual) portion (Fig. 3).

Group I. No radiographical changes were evident in this group.

Group II. About 30 per cent of the animals in this group showed evidence of disturbances. Formation disturbances were recognized on the basis of the irregular contour of the enamel surface. Disturbances in calcification were indicated by radiolucent areas in the lingual dentin of some of the lower incisors (Figs. 4-6). These areas were incremental in position. Corresponding areas were found to take an eosin stain in decalcified histological sections (Pl. 144, Figs. 1, 2) and showed prominent interglobular dentin in ground sections (Figs. 11, 12). The same zones were radiolucent in the Grenz X-ray plates. The latter permit microscopic examination of roentgenograms of ground sections and were kindly prepared for us by Dr. E. Applebaum of the Department of Oral Histology of the Columbia University Dental School. Three animals (5 per cent) showed fractures.

Group III. The changes in this group were similar to those in Group II but occurred in 50 per cent of the animals. Two animals (14 per cent) showed fractures.

Group IV. Each of the animals of this group showed pathological alterations. While the roentgenograms show very definite differences in the density of calcification they also indicate disturbances in formation. The irregular contour of the enamel surface,



the distortion of the incisors and the irregular width of the labial alveolar periosteum are seen in the majority of the animals (Figs. 7-10).

Each animal showed incremental radiolucent zones in the lingual dentin of the lower incisors. Many showed similar zones in the upper incisor and a few showed them in the enamel as well. The pulpal outline is as a rule very distinct and extends to the incisal edge. The prominent width of the pulp in the anterior portion of the incisor indicates an absence of deposition or a severe deficiency in the calcification of the dentin. Six animals (55 per cent) showed fractures in the incisors.

Elongation of teeth, extra- or intra-alveolar fractures, and blunted incisal bevels were found more frequently in this series than in the preceding groups (Figs. 7-10).

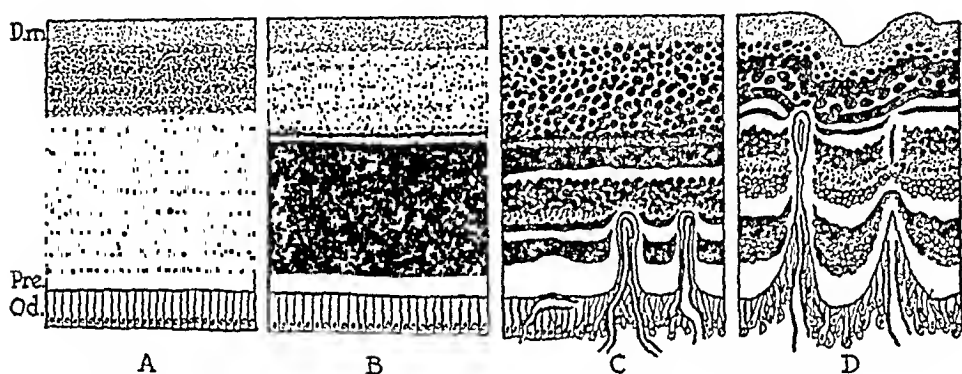
Two animals in this group show an exceptionally severe enamel hypoplasia in the proximal zone (Fig. 9). On first glance they remind one of the characteristic changes seen in long survival following hypophysectomy (Schour and van Dyke<sup>10</sup>). However, the changes differ from those seen in hypophysectomy in respect to the presence of a number of radiolucent incremental zones, and of elongation and the absence of the obliteration of the pulp.

#### HISTOPHYSIOLOGY OF THE INCISOR OF THE RAT

Since the incisors of the albino rat reflect with a high degree of accuracy and sensitivity the fluctuations in calcium and phosphorus metabolism, they have proved to be valuable indicators in studying the physiology of the parathyroid glands.

For a statement of the histophysiology and growth pattern of this tooth, the reader is referred to a brief report by Schour and Steadman.<sup>11</sup> In our present problem we are concerned primarily with the calcification pattern of the dentin. This tissue consists of a collagenous matrix in which lime salts are embedded. Both the apposition and the calcification of dentin proceed along a rhythmic incremental pattern at the rate of  $16\ \mu$  per 24 hours (Schour and Hoffman<sup>12, 13</sup>). There is a small time difference in which formation precedes calcification. As the dentin-forming cells recede pulpally and migrate distally, they continually form new dentin matrix, while the matrix formed on approximately the previous day is calcifying.

The matrix is calcified in the form of globules which follow the typical Liesegang ring formation; when one globule enters the sphere of influence of another a fusion occurs. Normally calcified dentin appears homogeneous because of complete fusion of a large number of spherical areas. But even in the normal condition, the successive layers of the dentin are not equally well calcified. Well calcified layers which stain more readily with hematoxylin alternate regularly and rhythmically with less well calcified layers which stain more readily with eosin. Thus there arises in the dentin a stratification which consists of pairs of dark and light increments (Text-Fig. 1, A). These pairs are of uniform width



Text-Figure 1. Schematic representation of the state of calcification of dentin of the incisor of the normal rat (A), and parathyroidectomized rat (B, C, and D).

A. Stippling represents normal dentin and shows the daily calcification and growth rhythm. The predentin (Pre) next to the normal odontoblasts (Od.) is even in width and white. The mantle dentin (D. m.) is located next to the enamel and is shown in fine dense stippling.

B. The stippled zone represents the preoperative dentin; the dark line followed by the narrow dotted zone represents the hematoxylin staining line and eosin zone in the dentin that is being formed and calcified at the time of and shortly after the operation. The black zone extending to the predentin is the heavy hematoxylin staining dentin found in the early parathyroprivic condition. B is representative of Group I.

C. The irregularly staining stratification zones in the parathyroprivic dentin of long survival are represented by the different size and spacing of globules (black). Note the greater width of the predentin and also the odontoblastic changes associated with the vascular inclusions. The dentin immediately adjacent to the inclusions like the predentin is white. C is representative of Group II.

D. The dentin surfaces bordering the enamel and the pulp are distorted. The incremental stratification zones are more irregular and show an arcade-like arrangement near the vascular inclusions. Odontoblastic changes are more prominent. D is representative of changes in Groups III and IV.

(approximately 16  $\mu$ ) and give the dentin its regular and characteristic incremental calcification pattern (Fig. 15, and Text-Fig. 1, A).

The latter presents different aspects in the various portions of the same tooth. There is an anteroposterior calcification gradient which expresses itself in the fact that the incremental pattern is most distinct in the distal portion of the incisor and difficult to recognize in the proximal portion.

There is also a pulpo-mantle calcification gradient which runs parallel with the course of the dentinal tubules from the pulpal surface to the mantle dentin. The dentin immediately next to the pulp consists of the newly formed matrix which is not yet calcified and is called the predentin. The latter is divided into early and late stages which stain differentially with hematoxylin and eosin. The transitional zone between dentin and predentin is known as the intermediate dentin which stains both with hematoxylin and eosin (Schour and Rogoff <sup>14</sup>). It is apparently in this intermediate zone that the dentin reaches its stage of mature calcification.

The normal process of secondary calcification which runs along the course of the dentinal tubules results in an increased calcium deposition which is in direct proportion to the age of the particular dentin increment, or its distance from the pulp. The dentin farthest away from the pulp is called the mantle dentin and appears to be most calcified.

When the globular calcification is incomplete, the dentin areas which are between the globules take an eosin stain and constitute interglobular dentin.

Occasionally a normal control will show an incremental layer which is conspicuous because of its preferential staining reaction with eosin. Such a reaction presents itself in the extremely sensitive dentin without any apparent outward manifestation in the habitus of the animal. This is due to the fact that calcification of the dentin is extremely sensitive to disturbances in calcium metabolism.

#### HISTOPATHOLOGY OF THE INCISOR OF THE PARATHYROID-ECTOMIZED RAT

Parathyroidectomy results in disturbances of calcification and growth. The essential character of the reaction varies with the

TABLE IV

*Dental Changes in Parathyroidectomized Albino Rats Arranged According to Experimental Groups I-IV*

Group	Experimental history		Gross changes	Fractures of incisors	Radiographic evidence of disturbances in calcification and formation	Histological changes			
	Number of rats	Survival period				Disturbances in calcification		Disturbances in formation	
						Enamel	Dentin	Enamel	Dentin
I (Short survival)	18	days 3-20	None	None	None	1. Sharp hematoxylin staining stripe in the dentin calcified about the time of the operation. 2. Postoperative dentin frequently stains deeply with hematoxylin	None	None	
II (Long survival)	57	39-181	Opaque enamel. White spots in enamel	In 5% of rats	In 30% of rats	1. Persistence of enamel matrix in distal portion. 2. Accentuated incremental line in matrix	1. Postoperative dentin interglobular. 2. Irregular and accentuated incremental stratification and zoning. 3. Vascular inclusions. 4. Calcospherites in distal pulp. 5. Predentin slightly wider than normal	Enamel epithelium disorganized in distal half. Occasionally isolated in connective tissue. Ameloblasts shortened or displaced by connective tissue	1. Vascular inclusions. 2. Irregular dentinopulpal and dentin-enamel border. 3. Odontoblasts crowded and disorganized near vascular inclusions
III (Fasting every 7th day)	14	105	As in Group II, but more consistent	In 14% of rats	In 50% of rats	More marked than in Group II. Enamel matrix stratified	More severe than in Group II	More severe than in Group II. Prominent atrophy of enamel epithelium. Enamel surface distorted	More severe than in Group II
IV (Repeated pregnancy and lactation)	11	120-360 (approximately)	As in Group III, but more marked; elongated	In 55% of rats	In 100% of rats	More marked than in Group III	More severe than in Group III	More severe than in Group III	2/3 normal width of dentin

postoperative survival. From the histological point of view, our material may be classified according to the same grouping that was obtained on the basis of the experimental history (Table IV, Text-Fig. 1).

*Group I. Changes During Brief Survival (3 to 20 Days)*

In this group the reaction to parathyroidectomy is an increase rather than a decrease in dentin calcification. The typical effect is well illustrated in Rat 55 which lived 8 days after the operation (Text-Fig. 1, B, and Figs. 1, 2). The postoperative dentin stains more deeply with hematoxylin than the preoperative dentin. At the border between the two there is a fine sharp line which stains deeply with hematoxylin and may be followed especially on the lingual portion by a narrow zone of eosin staining dentin (Fig. 2).

It has been experimentally demonstrated<sup>13</sup> that the rate of deposition of dentin in the rat incisor is approximately  $16\ \mu$  in 24 hours. This constant was used in our histological analysis. Measurements in the incisor of this animal and others of Group I indicate that the first hematoxylin stripe corresponds closely with the time of the operation.

In some animals of this group only the dentin of about the first 15 days after parathyroidectomy shows the deeper staining reaction, while the dentin laid down in the subsequent period shows a staining reaction which is normal and similar to that of the preoperative dentin.

*Group II. Changes During Longer Survival (More than About 35 Days)*

In this group the reaction to parathyroidectomy is one of progressive deficiency in the calcification and formation of dentin and enamel (Text-Fig. 1, C). The findings in Rat 29 are given in greater detail below and are illustrative of this group. Since replacement therapy with parathyroid extract was not initiated until 98 days after parathyroidectomy (Table II), we may consider this animal as parathyroprivic for 98 days. Four days following treatment the animal was sacrificed. A histopathological description of the upper left incisor is presented.

*Disturbances in Calcification:*

*Labial Dentin:* The disturbances in the calcification pattern of the dentin differ in intensity in different portions of the incisor. The proximal third of the labial dentin is characterized by considerable dispersion of interglobular dentin. The predentin is wider than normal and its border against the calcified dentin is invaginated by vascular inclusions. In the distal third the globular dispersion is masked by an accentuated stratification pattern which consists of an irregular alternation of deep hematoxylin staining areas and eosinophilic interglobular areas.

*Vascular Inclusions:* Vascular inclusions make deep penetrations, disturb the normal pattern, and often extend throughout the entire width of the dentin except for the portion next to the enamel (the mantle dentin). The incremental layers curve sharply and tend to follow the direction of the inclusions. The interruptions thus give an arcade-like appearance to the calcification pattern (Fig. 14) and are especially distinct because the dentin immediately adjacent to them is not calcified and stains like predentin. Concentric calcospherites are found in the most distal portions of the pulp (Fig. 16).

*Lingual Dentin:* In the lingual dentin the contrasting strata are especially outstanding, particularly in the distal third of the incisor. Here we have incremental eosin staining areas, some of which are as wide as  $150\ \mu$  alternating with narrower zones which stain deeply with hematoxylin. The position of the wide eosin staining areas corresponds with that of the incremental radio-lucent zones seen in the roentgenograms of the entire teeth and in the Grenz-ray pictures of the ground section of the incisors of parathyroidectomized rats of similar history. The entire dentin effect is one of irregular zoning of different densities in calcification superposed on the normal rhythmic stratification (Figs. 13, 14, 18-20).

*Fractures:* Three animals in this group show intra-alveolar fractures (Figs. 14, 17) which are as a rule transverse to the long axis of the incisor. A fracture which runs along the incremental pattern is shown in Figure 17. Incisal stress apparently split the tooth along an incremental zone which was poorly calcified and which was bordered by better calcified incremental layers on either side.

*Enamel:* The enamel space contains a remnant of the organic matrix at the level of the alveolar crest. The proximal third shows a sharply accentuated incremental line in the matrix. The position of this line indicates that it was produced approximately 2 weeks previous to death.

#### *Disturbances in Formation:*

*Dentin:* The formation of the dentin is interrupted by vascular inclusions and indentations, especially in its labial portion. The dentino-pulpal border is accordingly irregular (Plate 144, Fig. 1<sup>7</sup>). The odontoblasts are disorganized, especially in the distal third. Here they are more crowded and their long axis is not parallel with the direction of the dentinal tubules.

*Enamel:* The enamel epithelium is disorganized in the distal half. The epithelial projections are elongated, lose their parallel arrangement and are farther apart. The ganoblasts are shortened and together with the remaining epithelium are in some places displaced by intra-epithelial cyst-like formations. Small isolated isles of epithelium are found in the labial alveolar periosteum.

Near the alveolar crest the enamel epithelium is completely absent and replaced by connective tissue which is filled in part with an eosin staining fluid. At this point the organic enamel matrix is still present. The labial alveolar periosteum is wider than normal.

#### *Group III. Changes in Animals that Survived 105 Days and were Subjected to Fasting on Every 7th Day*

The findings in Rat 50 are illustrative of this group (Text-Fig. 1, D). This animal was permitted to live for 105 days following operation. There was a deviation from the usual feeding routine in so far as the animal was submitted to fasting every 7th day. The histopathological changes of the upper left incisor are described below.

#### *Disturbances in Calcification:*

*Dentin:* The changes are more severe than those observed in Group II. The stratification is markedly accentuated (Fig. 13). The dentin is interrupted by undercalcified areas surrounding vascular inclusions. The predentin is poorly calcified and its width in the proximal labial third is 40 $\mu$ .

Calcospherites similar to those seen in Figure 16 were found in the most distal portions of the pulp of the lower incisors. Their presence in the upper incisors could not be determined because the sections did not pass through the midsagittal plane in the distal portions.

*Enamel:* Disturbances in the enamel calcification are associated with atrophic changes in the enamel epithelium. Proximally the outer enamel surface is distorted and the enamel matrix is stratified (Fig. 12). A portion of the enamel matrix persists as far as the gingival crest. Here, too, the surface is distorted (Fig. 13).

#### *Disturbances in Formation:*

*Dentin:* In the distal region the dentin surface shows distortions at levels that also show corresponding disturbances in the enamel. Formation is interrupted by vascular indentations and inclusions. The changes in the odontoblasts are similar to those seen in Rat 29.

*Enamel:* The enamel epithelium exhibits a series of severe atrophic changes. Proximally some of the cells have completely degenerated and enamel globules are found in the cellular débris. Here the enamel matrix is, for the most part, replaced by an accumulation of eosin staining fluid. In the middle third the epithelium is folded and has retrogressed to a low cuboidal type. Occasionally masses of closely packed atrophic enamel epithelium protrude into the enamel space or extend into the labial alveolar periosteum. The enamel at these points is undercalcified, as evidenced by the persistence of enamel matrix as seen in Plate 144, Figure 4.<sup>7</sup> This reaction repeats itself distally (Fig. 13).

In this group the alveolar and jaw bones show an intense staining of the cementing lines and the lining of the Haversian canals with hematoxylin. A similar reaction was also observed in Group II.

#### *Group IV. Changes in Animals that Survived for a Period of 4 Months to 1 Year and were Subjected to Repeated Pregnancies and Lactations*

The histological changes in this group confirm the severe disturbances seen in the roentgenograms (Figs. 7-10). The alterations are much more severe and intense than in those of Group III (Text-Fig. 1, D).



*Disturbances in Calcification and Formation:*

The calcification disturbances in the dentin differ from those described in Groups II and III in respect to their increased intensity but are similar in type. The enamel shows prominent and persistent incremental stratification (Fig. 12). The organic matrix is found to persist in the majority of animals throughout the extent of the enamel space and adheres to the exposed portion of the incisors.

The disturbances in formation are relatively more prominent than the increased disturbances in calcification. The enamel surface and the dentino-enamel and dentino-cemental borders are extremely wavy or distorted. In 2 animals there is a severe buckling up of the enamel and the dentin in the proximal portion (Fig. 9) which reminds one of similar distortions seen characteristically in hypophysectomy.

In a number of animals the dentin at the distal end is half or two-thirds the normal width. Ankylosis of the labio-alveolar periosteum with the dentin is found in 1 animal. Calcospherites in the pulp are seen more frequently in this group than in the previous groups. The enamel epithelium frequently shows early premature atrophy.

## DISCUSSION

*Comparison of Our Findings with Those Reported in the Literature*

Our findings on the dental changes in the incisor of the parathyroprivic rat differ from those reported in the literature particularly by Erdheim<sup>1</sup> and Toyofuku<sup>2</sup> in the following respects:

1. The rate of eruption measured in 10 rats was normal. Gottlieb<sup>15</sup> reported retarded eruption.

2. In early survival periods (up to about 20 days) the parathyroprivic dentin shows denser calcification than the preoperative dentin. Previous investigators reported an immediate impoverishment or absence in calcification (Erdheim,<sup>5</sup> Toyofuku,<sup>2</sup> von Spreter<sup>16</sup>).

3. For any given similar period of survival longer than about 20 days our findings show disturbances of a type similar to that reported by previous investigators but of lesser severity.

4. Our findings show less prominently the rachitic-like changes

that are emphasized in the literature in respect to the presence of wide predentin and a transitional interglobular dentin zone (Toyofuku,<sup>2</sup> Erdheim<sup>6</sup>).

### *The Possible Basis for the Difference in Results*

In evaluating the possible reasons for the difference in results in the various investigations the following factors should be considered: completeness of operation, stock of animals, age at operation and diet.

It is justifiable to assume that our surgical removal was as complete as was the case in previous investigations. Our parathyroidectomy was as complete as is experimentally possible. The histological analysis of all the tissue removed at the time of the operation and the blood calcium were used as an index of the completeness of the removal. Histological studies showed the presence of accessory tissue in less than 10 per cent. These results confirm earlier findings (Hoskins and Chandler<sup>17</sup>) and indicate that accessory parathyroids do not vitiate results in a large and well controlled series of experiments.

Our animals were of the Wistar strain, which has become a standard for experimental purposes, but this is not the probable reason for the differences in results. The age at operation is not a significant factor in the difference in effects because our experimental series included animals of the same ages used by previous investigators; moreover, most of the animals were operated on during the period of most marked susceptibility to parathyroidectomy (Hoskins<sup>18</sup>).

The differences can be explained largely on the basis of dietary factors. Our basal diet was adequate in calcium (1.41 per cent) and phosphorus (0.98 per cent) and in vitamin D. The diet employed by Erdheim and Toyofuku was very likely deficient in vitamin D and calcium. Shelling<sup>19</sup> points out that the dietary knowledge was incomplete at the time of Erdheim's experiment and that the diet of Erdheim's animals consisted chiefly of bread.

Erdheim<sup>6</sup> concluded in his work on rickets and parathyroids that the dental changes in rickets were similar to those in parathyroidectomy. This conclusion is not supported by our findings. It is likely that the basal diet of Erdheim's parathyroidectomized rats was at least slightly rachitogenic. Shelling<sup>20</sup> demonstrated

the varying effects of changes in diet in their effect in fracture healing in parathyroidectomized animals. Shelling's findings differed from those of Erdheim apparently because of a difference in the diets that they employed.

Von Korenchevsky<sup>21</sup> states that the macroscopic changes in the teeth of his parathyroprivic animals were not as severe as those observed by Erdheim. Von Korenchevsky found the most profound changes in animals that had a deficiency or lack of fat soluble vitamin A. In 1922 he did not realize that on the basis of his diet this also meant vitamin D deficiency. He was unable to confirm Erdheim on the rickets-producing effect of the removal of the parathyroid. He points out that "diet is an all important factor which can by itself produce profound changes in the skeleton and comparatively little, if any, attention was paid to the diet by the majority of the above investigators," (referring to Erdheim and others).

Von Spreter<sup>16</sup> does not give the diet employed in his work published in 1936. In one of his reports<sup>22</sup> dealing in part with parathyroidectomy, he states that the diet consisted of grain plus various breads soaked in water. It appears that the findings of previous investigators were a result of at least slight nutritional deficiencies superposed on parathyroidectomy.

It is possible that slight deficiencies in the diet are insufficient to produce noticeable effects in normal animals but may contribute to more marked effects when combined with parathyroidectomy.

The available data indicate clearly that the dietary effects exert significant influence on the course of events in the parathyroprivic animal.

*The Importance of the Survival Factor: Possible Basis for the  
Denser Calcification of the Parathyroprivic Dentin  
Formed During Short Survival Periods*

The findings show that a correlation exists between the duration of the survival period and the histological response. Thus, in our group of animals surviving from 3 to 20 days the evidence indicates an increase in the density of dentin. Survival beyond this period is associated with a progressive impoverishment in dentin calcification and a progressively accentuated alternation of over- and under-calcification.

The data available in the literature indicate a retention of calcium in the body particularly in early survival periods of parathyroidectomy. The occurrence of hypocalcemia is well established. In a complete analysis of calcium and phosphorus metabolism in the parathyroidectomized rat, Bülbring<sup>23</sup> reports an increased calcium and phosphorus retention in early parathyroidectomy which lessens as the survival period progresses. The fact that hypercalcification of dentin occurs in the early survival periods after parathyroidectomy would seem to be in agreement with this observation. Bülbring, however, points out that the retained calcium is deposited in the soft tissue rather than in the bone. Our evidence seems to indicate that given a diet that is adequate in calcium, phosphorus and vitamin D, there is retention also in dentin. This harmonizes with Erdheim's calcio-protective law, according to which the growing dentin is a tissue which is given special protective preference in cases of disturbances in calcium metabolism. Furthermore, calcified dentin, unlike bone, is not subject to calcium withdrawal (Albright, Aub and Bauer<sup>24</sup>).

The lessened retention of calcium and phosphorus in later periods and the establishment of a relatively more stationary level of lowered blood calcium may account for the characteristic and better known calcification deficiencies in dentin during longer periods of survival.

### *The Effect of Fasting*

Fasting on every 7th day resulted in an increase in the severity of the histopathological changes. The most prominent effect was an accentuation in the stratification as shown in Figure 13.

Measurements of the intervals between the prominent eosin staining stripes did not correspond closely with the intervals between the fasting periods, as might have been expected. It appears that the rhythmic factors that make for the characteristic accentuated fluctuation of calcification in the dentin following parathyroidectomy are more basic and are not promptly, if at all, modified by short fasting periods.

It is probable that in our experiment fasting exerted its influence primarily through the resulting decrease in the intake of calcium and other food factors that promote calcification.

*Effects of Repeated Pregnancies and Lactations*

The most severe histopathological alterations were found in the series of rats (Group IV) that were subjected to repeated pregnancies and lactations. The greater drain on calcium is probably chiefly responsible for the more severe reaction. Our findings do not support the view that the greater demand for calcium resulted in a withdrawal of calcium from the teeth. It is more likely that the calcium needed by the normal growing increments of enamel and dentin was much less available under the repeated pregnancies and lactations than under conditions of parathyroidectomy alone.

If calcium withdrawal were responsible for the disturbed calcification in the incisors, a similar disturbance should be recognizable in the molars. This was not the case. The latter showed normal calcification because they had completed their formation and calcification before or soon after parathyroidectomy and subsequent modifications did not occur.

It is also possible that the combined effect of parathyroidectomy and repeated pregnancies and lactations results in a disturbance of the interrelations of the endocrines and thereby aggravates the changes that are characteristic of parathyroidectomy alone.

The greater incidence of fractures in this group can be correlated with the more severe fluctuations in the calcification pattern of the dentin. It appears that the extreme fluctuations of excessive calcification and lack of calcification, rather than the lack of calcification alone, are responsible for the readiness of the parathyroprivic dentin to fracture.

*Possible Factors in the Causation of Developmental Changes*

Developmental changes in the incisor of the parathyroprivic rat as a result of long survival periods were found chiefly in the enamel organ and also involved to a lesser extent the odontoblasts. These cellular injuries, as well as the vascular inclusions, tend to be localized and are associated with the local disturbances and interruptions of the calcification pattern that is characteristic for the parathyroprivic condition.

The fact that the dentin showed a very narrow width in a number of the rats of Group IV suggests that the life span of the odontoblasts was shortened considerably in those cases.

Variations in the calcium and phosphorus content of the in-

ternal medium of the cells over a prolonged period may very likely account for the atrophic changes. There is also the possibility that the absence of the parathyroid hormone over a long interval might exert a direct effect on the cell metabolism or on growth. The ganoblasts seem to be especially affected.

*The Sensitivity of the Reaction of Dentin to Various Disturbances in Calcium Metabolism: The Calciotraumatic Ring*

The dentin that was formed and calcified during and immediately after the operation shows in longitudinal sections a fine sharp line or sometimes a double line which stands out by its deeper and more distinct staining with hematoxylin (Figs. 1, 2). In transverse sections this line takes the appearance of a ring. This ring is not seen in animals of long survival because the dentin formed at the time of the operation has been abraded. This ring is not characteristic of parathyroidectomy alone. It has also been found subsequent to adrenalectomy<sup>14</sup> and hypophysectomy (unpublished data) in the dentin forming and calcifying at the time of the operation.

A similar acute response is also found to be associated with the effects of injections of parathyroid extract<sup>9</sup> and sodium fluoride.<sup>25</sup> The immediate primary hypocalcified zone following the injection of sodium fluoride is often readily demarcated from the preexperimental dentin by a fine narrow hematoxylin staining line which facilitates the recognition of the starting point for measuring the width of the postoperative dentin.

This acute dentin response offers an interesting problem for analysis. It seems to be an expression of a shock to calcium metabolism. This is perhaps induced by the trauma incident to surgery, ether anesthesia, or acute endocrine disturbances. It may be an overcalcification effect associated with a very brief period of arrested growth (Harris<sup>26</sup>). Regardless of the particular etiology of this reaction, we may consider the latter as an experimentally or otherwise induced hypercalcification effect in the dentin. The ring itself might be referred to as the calciotraumatic dentin ring. It illustrates in an accentuated manner the normal dual character of the calcification process of dentin which consists of a rhythmic alternation of dense and less dense mineralization.

An analogous macroscopic condition has been observed in the

roentgenograms of long bones which register transverse lines of arrest<sup>26</sup> or scars<sup>27</sup> in response to various acute disturbances.

### *Variations in Calcification Disturbances Within the Same Incisor*

Erdheim's calcioprotective law emphasizes the fact that disturbances in calcium metabolism do not affect different tissues in the same manner or to the same degree. Our findings indicate that this generalization is also applicable to the different teeth of the same animal and to different portions of the same tooth. The experimental disturbances in the dentin calcification were more severe in the upper than in the lower incisors and were more evident in the lingual than in the labial portions.

It is interesting that even in the most severe alteration in the calcification of the dentin, the portion immediately adjacent to the enamel or cementum (mantle dentin) is not affected. There appear to be localized factors that modify to a limited extent the effect of systemic conditions.

### *The Validity of Hematoxylin as an Indicator of Calcification*

The question of the validity of the hematoxylin-eosin stain used in decalcified sections as an indicator of calcification was discussed in detail in a previous report (Schour and Ham<sup>28</sup>). In this study confirmation of our interpretations on the basis of the staining reaction was obtained by the roentgenograms. Incremental eosin staining zones (Plate 144, Fig. 2) were found to be radiolucent in the roentgenograms (Figs. 1, 4, 7, 8). We have obtained additional confirmatory evidence by the use of Grenz X-rays of ground sections that were kindly taken for us by Dr. E. Applebaum. Further and more conclusive evidence awaits the application of microhardness tests, microchemical analysis and possibly X-ray diffraction studies.

It is hoped that in the near future improved methods and facilities for analysis will clarify further the important problem of interpreting different quantitative and qualitative degrees of calcification in the hard dental tissues.

### SUMMARY AND CONCLUSIONS

The effect of parathyroidectomy on the incisor of the albino rat was studied in 100 rats (3 to 360 days after operation) and 27 controls, in respect to gross changes (including roentgenograms

and eruption rates) and microscopic alterations. Eighteen rats were allowed to survive only 3–20 days (Group I). Fifty-seven rats survived a period of 39 to 159 days (Group II). Fourteen rats survived 105 days and were subjected to fasting every 7th day (Group III). Eleven rats survived approximately 4 months to 1 year, and were subjected to repeated pregnancies and lactations (Group IV).

The incisor of the parathyroidectomized rat shows disturbances in calcification and in development. The essential character of the reaction varies with the length of the survival period.

Group I. In short survivals up to 20 days the postoperative dentin indicates denser calcification than normal and is demarcated from the preoperative dentin by a distinct ring, which stains deeply with hematoxylin. This ring, which is formed at the time of the operation and which is also found in other related experimental conditions, appears to be an acute response to a shock to calcium metabolism and may be referred to as the calciotraumatic ring. The denser calcification of the postoperative dentin in this group may be a result of the calcium retention that is reported to persist during the early survival period.

Group II. In longer survivals the changes consist of defective calcification and defective formation of enamel and dentin. The severity of the changes increases with the increase in time of survival. The most characteristic change consists of an aberration in dentin calcification. The dentin shows an irregular and accentuated alternation of zones of different densities in calcification. This severe fluctuation, rather than lack of calcification, is responsible for the readiness of the parathyroprivic dentin to fracture.

Group III. The histological changes are aggravated by fasting on every 7th day.

Group IV. The most severe alterations were found in animals that were subjected to repeated pregnancies and lactations. Histological examination shows no evidence of calcium withdrawal from the calcified tissues of the teeth.

Our findings differ from those reported in the literature in the following respects: dense calcification of dentin during the early survival period (Group I); less severe changes for same period of survival (Group II); and a less prominent rachitic-like width of predentin. These differences may be ascribed chiefly to a difference in diet.



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## DESCRIPTION OF PLATES

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### PLATE 142

Fig. 1. Microphotograph of a longitudinal section from the midregion of the upper right incisor of Rat 55 which was killed 8 days after parathyroidectomy. Note the deep hematoxylin staining line (1) at the level of the dentin which was calcifying at the time of operation and which separates the preoperative dentin (D. pr.) from the postoperative dentin (D. po.). The latter takes a deeper stain with hematoxylin. Measurements of the distance of the line 1 from the predentin show that the position of the line corresponds closely with the position of the dentin that was being calcified about the time of the operation. En. m. = enamel matrix; L. a. p. = labial alveolar periosteum; Od. = odontoblasts.  $\times 84$ .

Fig 2. Microphotograph of a longitudinal section from the midregion of the lingual half of the upper right incisor of the same animal as in Figure 1. The deeper hematoxylin staining line (1) is followed pulpally by a

lighter staining zone (z) and separates the preoperative (D. pr.) from the postoperative dentin (D. po.). The calcification is modified after the operation, as is evidenced by the deeper staining reaction. Cem. = cementum; Od. = odontoblasts; P. d. m. = periodontal membrane.  $\times 84$ .

Fig. 3. Roentgenogram of right half of the head of an adult control rat. Note the smooth curvature of the tooth surfaces. The distal third or half of the tooth is radiopaque while the radiolucent pulp can be seen more posteriorly. Figures 3-10 are natural size.

Fig. 4. Roentgenogram of the upper and lower incisors of parathyroidectomized Rat 120 of Group II. Note the irregular contour of the labial outline of the upper incisor which is found in about 30 per cent of the animals in this group. The radiolucent appearance of the pulp persists as far as the alveolar crest. In the lingual dentin of the lower incisor, near the alveolar crest, there is an incremental radiolucent zone between two radiopaque borders. This zone corresponds to the eosin staining areas seen in the microscopic sections. The lower incisor is elongated because its antagonist had been fractured.

Fig. 5. Roentgenogram of Rat 10A of Group II. The upper incisor shows an intra-alveolar fracture and a wide pulp. In the lower incisor the radiolucent appearance of the pulp extends to the incisal edge.

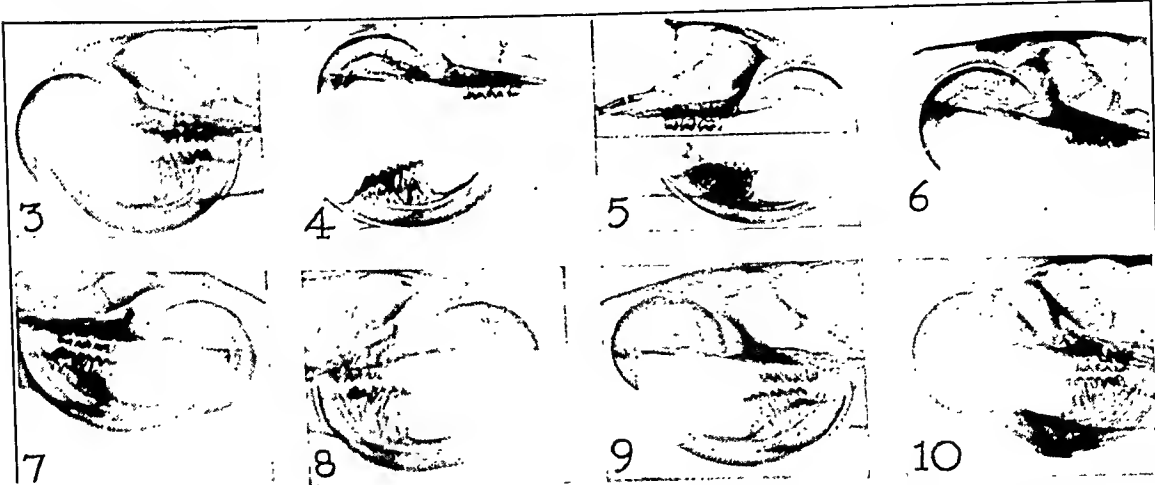
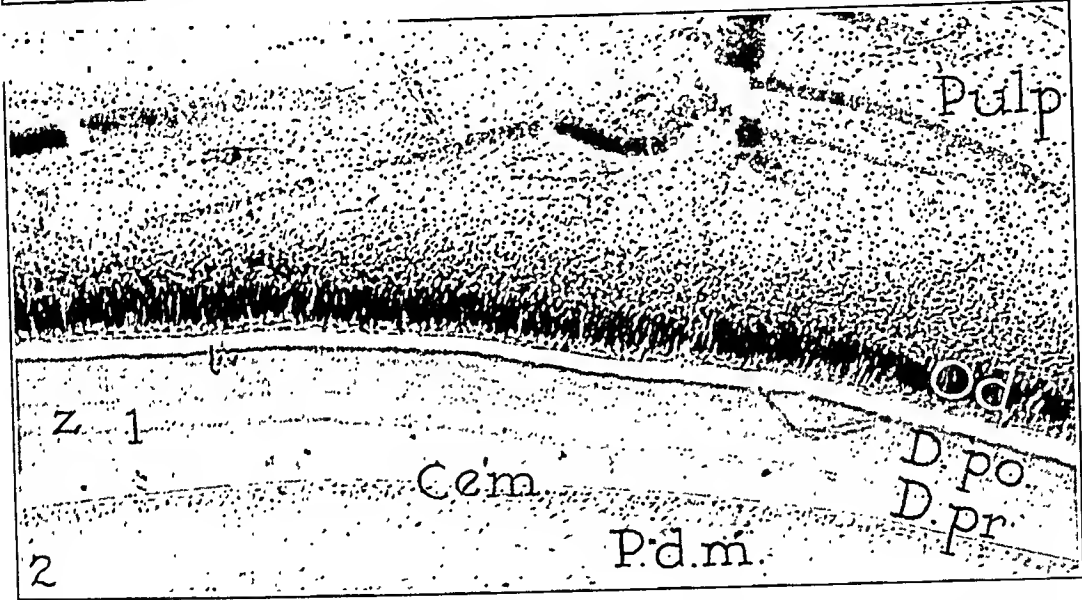
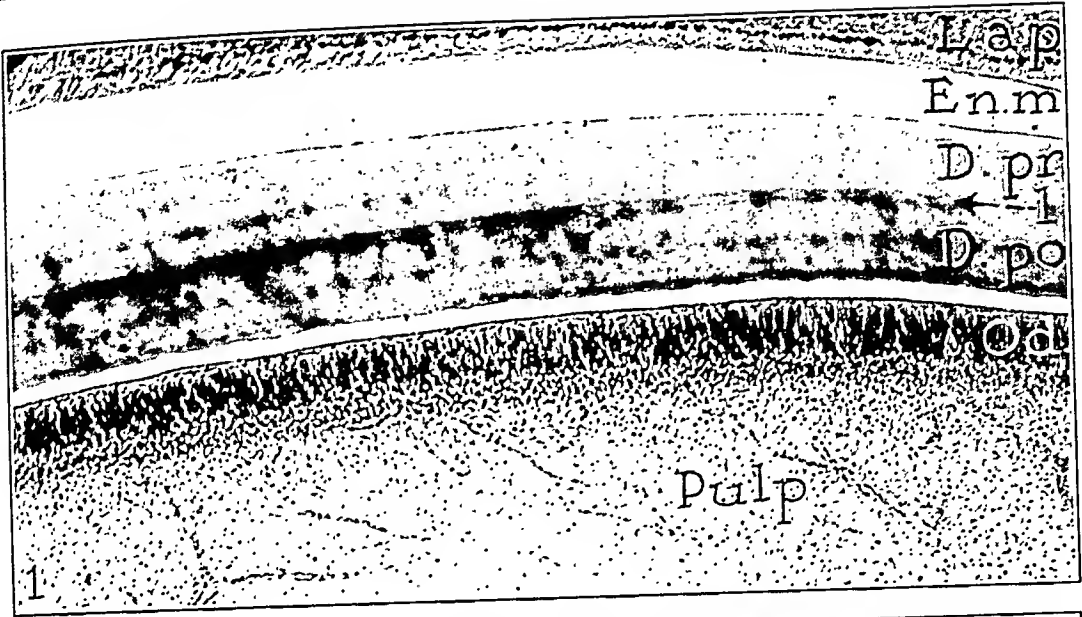
Fig. 6. Roentgenogram of the upper right incisor of Rat 119 of Group II. Note the irregular contour of the enamel surface and the extent of the pulp to the incisal edge. The notch in the enamel near its distal end represents the marking filed into the tooth for the purpose of measuring the rate of eruption.

Fig. 7. Roentgenogram of the left half of the head of Rat 709 of Group IV. Note in the upper incisor the elongation; the irregular contour of the enamel surface; the irregular width of the labial alveolar periosteum; and the abnormal width of the pulp and the persistence of its radiolucency beyond the alveolar crest. The lower incisor shows an incremental radiolucent zone in the distal half of its lingual portion and an intra-alveolar fracture.

Fig. 8. Roentgenogram of the right half of the head of Rat 704 of Group IV. Note in the upper incisor the irregular enamel surface, the irregular width of the labial alveolar periosteum and the fracture. Note in the lower incisor the intra-alveolar fractures and the lingual incremental radiolucent zone in the fractured segment.

Fig. 9. Roentgenogram of the left half of the head of the same rat as in Figure 8. Note in the upper incisor the distortion of the curvature and the severe enamel hypoplasia in the basal area. The pulp is radiolucent only in the proximal half. The lower incisor is thickened in the proximal end of the labial surface and shows a fracture.

Fig. 10. Roentgenogram of the right half of the head of Rat 712 of Group IV. The changes are characteristic of Group IV. Note the elongation of the upper incisor through the lack of function subsequent to the fracture of the lower tooth. Note the perforation of the palate at the distal end of the upper incisor which almost meets its proximal beginning. The dentin shows radiolucent zones. The lower incisor shows a thickening of its proximal labial end.





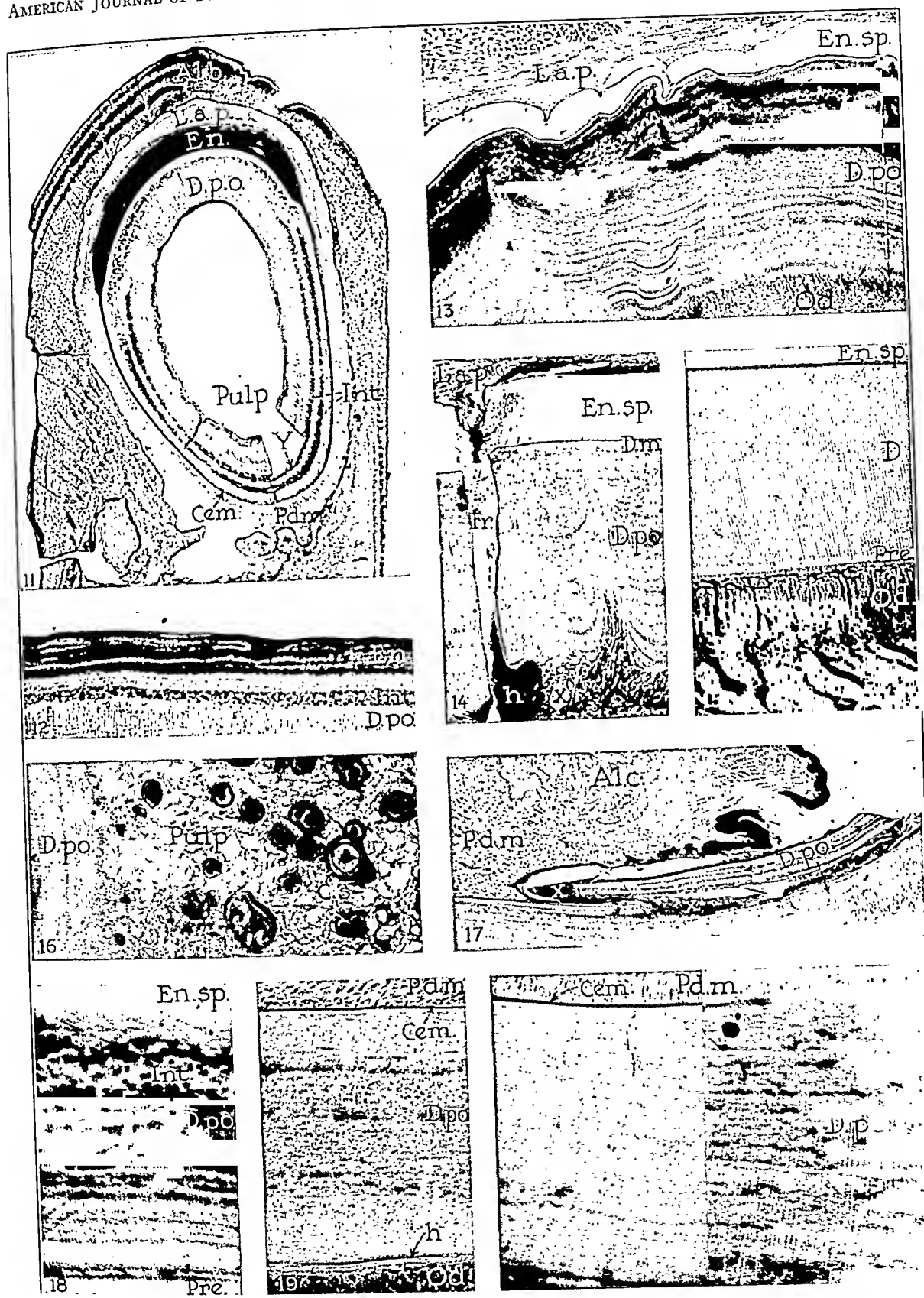
- Fig. 11. Microphotograph of a transverse ground cross section of the lower left incisor of Rat 64 which survived 157 days after parathyroidectomy. It received 1 injection of parathyroid extract 3 days before death. Note the two prominent concentric rings of interglobular dentin (Int.). The inner ring is absent where the dentin (D. po.) is covered by the enamel. The missing dentin was fractured in grinding along the poorly calcified incremental zone (Y.). The enamel (En.) shows a lack of homogeneous calcification. The labial alveolar bone (Al. b.) at the left shows interesting rhythmic calcification zones. L. a. p. = labial alveolar periosteum; P. d. m. = periodontal membrane; Cem. = cementum. Pulp torn out in grinding.  $\times 23$ .
- Fig. 12. Microphotograph of a longitudinal ground section from the midregion of upper incisor of Rat 709 which was parathyroidectomized and subjected to repeated pregnancies and lactations. Note the wavy surface and prominent incremental stratification in the enamel (En.) and the zone of interglobular dentin (Int.) in the postoperative dentin (D. po.).  $\times 60$ .
- Fig. 13. Microphotograph of a longitudinal section from the distal portion of the labial dentin of the upper left incisor of Rat H50 which lived 105 days after parathyroidectomy and was subjected to fasting every 7th day. Note the wave-like disturbance of the dentino-enamel junction and the enamel surface. The parathyroprivic dentin (D. po.) shows the typical irregular calcification zoning. The enamel epithelium is severely atrophied. L. a. p. = labial alveolar periosteum; En. sp. = enamel space. Od. = odontoblasts.  $\times 34$ .
- Fig. 14. Microphotograph of a longitudinal section from the midregion of the labial dentin of the upper right incisor of Rat 116 which lived 120 days after parathyroidectomy and was given 1 administration of calciferol 24 hours before death. Note the downgrowth of the labial alveolar periosteum (L. a. p.) and the hemorrhage in the pulp (h) at the fracture (Fr.) showing that the latter is not an artefact; the alternate zones of varying degrees of disturbances in calcification; and the bending of the zones at the sides of the vascular inclusion (V. i.). En. sp. = enamel space; D. m. = mantle dentin; D. po. = postoperative dentin; X = cyst in odontoblastic layer.  $\times 62.5$ .
- Fig. 15. Microphotograph of a longitudinal section from the labial dentin of the upper incisor of a control rat. Note the uniform density of calcification and the normal calcification rhythm. Compare with the parathyroprivic dentin in Figures 13 or 14 of this plate. En. sp. = enamel space; D = dentin; Pre = predentin; Od. = odontoblasts.  $\times 100$ .
- Fig. 16. Microphotograph of a longitudinal section from the incisal portion of the pulp of the upper incisor of Rat 119 of Group II. Note the complete atrophy of the pulp and the numerous calcospherites. D. po. = postoperative dentin; C. s. = calcospherites.  $\times 115$ .

Fig. 17. Microphotograph of a longitudinal section from the incisal portion of the lingual dentin of the upper right incisor of Rat 126 which lived 133 days after parathyroidectomy and was given 1 injection of parathyroid extract 240 hours before death. The dentin fractured along an incremental line of disturbed calcification. Contrast with the type of fracture seen in Figure 14. Note the proliferation of the alveolar bone at the crest (Al. c.); the downgrowth of epithelium along the fractured surface which faces the periodontal membrane and the cellular debris collected at X. D. po. = postoperative dentin; P. d. m. = periodontal membrane.  $\times 22.5$ .

Fig. 18. Microphotograph of portion of a longitudinal section from the midlabial dentin of Rat H53 of Group III. Note the interglobular dentin (Int.) and the calcification zones of different widths and densities in the parathyroprivic dentin (D. po.). En. sp. = enamel space; Pre. = predentin.  $\times 90$ .

Fig. 19. Microphotograph of a longitudinal section from the midregion of the lingual dentin of the lower right incisor of Rat 113, which lived 120 days after parathyroidectomy and was given 1 administration of calciferol 24 hours before death. D. po. = dentin laid down previous to the administration. Note the alternate zones of varying degrees of calcification disturbances, particularly the uncalcified zone formed preceding the time of administration. Note the very narrow hematoxylin staining zone (h) immediately next to the narrow predentin. This zone was produced by the administration of calciferol. Cem. = cementum; Od. = odontoblasts; P. d. m. = periodontal membrane.  $\times 100$ .

Fig. 20. Microphotograph of a longitudinal section from the lingual dentin of Rat H40 of Group III. Note the prominent zoning of eosin or hematoxylin staining dentin and the irregularity in width and staining reaction of these zones. Fasting on every 7th day tended to accentuate the zoning effect. Cem. = cementum; P. d. m. = periodontal membrane; D. po. = postoperative dentin. The right and left portion of the figure show one field but the photographs were taken with different filter and focus to show interglobular texture and irregular stratification pattern.  $\times 180$ .







## II. THE EFFECT OF PARATHYROID EXTRACT AND CALCIFEROL ON THE INCISOR OF THE RAT

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This study is concerned with the evaluation and comparison of the effects of parathyroid hormone and of calciferol (vitamin D) on the incisors of parathyroidectomized rats.†

### REVIEW OF LITERATURE

Von Spreter<sup>1</sup> administered variable doses of parathyroid hormone to 20 rats within 12 days, or less, after parathyroidectomy. He found that a minimum of 20 to 24 (Collip) units of parathyroid extract (Lilly) was necessary to produce complete calcification of the "dentinoid" tissue and that the effect of a single injection persisted for 12 to 24 hours. Schour and Ham,<sup>2</sup> and Schour, Tweedy, and McJunkin<sup>3</sup> reported the effects of variable, single or multiple, doses of vitamin D, and of parathyroid hormone on the serum calcium and incisors of normal rats. They found that a single effective dose of either vitamin D or parathyroid hormone produced in the dentin a primary hypocalcified stripe which was followed by a hypercalcified stripe.

Von Spreter<sup>4</sup> treated 3 parathyroidectomized rats with daily doses of 150 rat units of irradiated ergosterol, and 3 parathyroidectomized rats with daily doses of 5000 rat units of irradiated ergosterol. In each case the administration was begun after the appearance of tetany. The doses of 150 rat units of irradiated ergosterol had no effect. The doses of 5000 rat units prevented and healed the parathyroprivic defects in the teeth, without metastatic calcification or other symptoms of overdose.

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## METHODS AND MATERIAL

This report is based on observations of 59 parathyroidectomized albino rats. Twenty-nine of these animals received from 1 to 4 injections of either Lilly's parathyroid extract or of the extract prepared by one of us (W. R. T.). The total dosage ranged from 10 to 274 (Collip) units (Table I). Single doses of 46,000 to 644,000 international units of calciferol were administered orally to 30 animals (Table II). The calciferol was in solution in Mazola

TABLE I

*Data on 29 Parathyroidectomized Rats Treated with Parathyroid Extract,  
Arranged According to Survival Period*

Rat No.	Post-operative survival to injection time	Weight at injection	Collip units	Number of injections	Interval between first injection and death	Calculated survival
	days	gm.	per 100 gm.		days	days
2	66	..	50	1	3	2.6
7	66	158	50	1	3	2.6
3	66	247	50	1	3	2.8
1	66	224	50	1	3	2.9
37	20	145	10	1	3	2.9
38	20	147	10	1	3	3.0
64	157	215	25	1	3	3.0
32	98	290	25	1	4	3.7
66	157	163	50	1	4	3.8
96	157	274	25	4 <sup>1</sup>	4	3.9
120	125	220	25	4 <sup>1</sup>	4	3.9
48	47	155	25	1	4	4.0
29	98	257	25	1	4	4.1
904*	83	167	10	1	4	3.7
907*	83	to	10	1	4	3.7
903*	83		10	1	4	3.8
905*	83		10	1	4	4.2
901*	83		10	1	4	4.3
906*	83	298	10	1	4	4.4
119	125	241	25	4 <sup>1</sup>	5	5.0
100	140	190	50	1	8	8.2
101	140	240	50	1	8	8.4
30	98	298	25	1	9	8.8
67	39	156	50	1	9	8.9
112	135	228	25	2 <sup>2</sup>	11	10.8
108	135	215	25	2 <sup>2</sup>	11	11.3
111	135	188	25	2 <sup>2</sup>	11	11.6
68	39	173	50	1	17	17.1
47	47	160	100	1	17	17.3

\* History of animals marked 900 or above was available for entire group only.

<sup>1</sup> Interval between injections, 1 day.

<sup>2</sup> Interval between injections, 3 days.

TABLE II

*Effect of Calciferol on the Serum Calcium of Parathyroidectomized Rats,  
Arranged According to Survival Period After Administration*

Rat No.	Post-operative survival to first administration	Weight at administration	Total dose in I.U. ( $\times 10^3$ )	Post-administration survival	Serum calcium (just before sacrifice)
	days	gm.		hrs.	mg./100 cc.
*118	120	176	368	24	12.21
113	120	233	552	24	12.21
116	120	267	644	24	12.21
					Pooled sample
1A	58	148	184	48	13.60
3A	58	152	276	48	13.60
5A	57	190	378	48	13.60
					Pooled sample
91	159	207	460	72	16.04
92	159	217	460	72	16.04
93	159	186	368	72	16.04
					Pooled sample
13A	56	159	276	96	14.18
14A	56	182	368	96	14.18
17A	55	124	184	96	14.18
					Pooled sample
25A	42	175	368	120	13.18
*26A	42	170	276	120	13.18
27A	42	124	184	120	13.18
					Pooled sample
4A	57	207	460	144	11.63
6A	56	217	460	144	11.63
10A	56	186	368	144	11.63
					Pooled sample
125	133	168	368	168	9.23
*124	133	142	184	216	7.79
126	133	165	276	240	8.81
					Individual sample

*Administration of Small Doses of Calciferol to Parathyroidectomized Rats*

*1B	49	150	46	66	10.46
†3B	49	141	46	66	
*4B	49	220	115	66	
5B	49	249	138	66	13.37
6B	48	220	115	66	11.34
†7B	48	282	161	66	11.44
*8B	48	175	92	66	
†9B	48	182	92	66	11.19
10B	48	170	69	66	11.19
					Pooled sample

\* These rats were not studied histologically.

† In the cases of animals 3B, 7B, and 9B, the serum calcium 17 hours after injection was 8.62, 9.60 and 9.77 mg. per cent respectively.

oil. The untreated parathyroidectomized rats included in the preceding report were used as controls. Therapy was instituted at 20 to 159 days following the operation and the animals were sacrificed 3 to 17 days after the administration of calciferol or the last injection of parathyroid hormone. The histological technique was the same as that used in the preceding report.<sup>5</sup>

### CHEMICAL FINDINGS

Serum calcium values were obtained immediately after sacrifice of each parathyroidectomized animal to which calciferol was administered (Table II). There appears to be a correlation between the serum calcium values, the duration of the postadministration

TABLE III

*Effect of Calciferol on the Serum Calcium of Normal Rats*

Rat No.	Weight at administration	Total dose in I.U. ( $\times 10^3$ )	Post-administration survival	Serum calcium (just before sacrifice)
	<i>gm.</i>		<i>hrs.</i>	<i>mg./100 cc.</i>
1	133	184	48	15.23
3	173	368	48	15.23
5	139	184	48	15.23
				} Pooled sample
2	156	276	48	15.35
4	213	460	48	15.35
				} Pooled sample

period and the dosage. The serum calcium tends to rise during the first 3 days following the injection and then begins to decline thereafter. The level and rapidity of the rise appears to increase with the dosage. In the series of 9 parathyroidectomized rats that were given one-fourth the dosage of calciferol and in which serum calcium levels were obtained 17 hours and 66 hours after the administration, the elevation of serum calcium was not so pronounced. Table III also indicates the serum calcium values obtained in a series of 5 normal rats that were given calciferol and were allowed to live 48 hours. The calcium level is higher than in the corresponding group of parathyroidectomized rats that were given approximately the same dosage per 100 gm. of body weight and killed at the same time interval.

## HISTOLOGICAL FINDINGS

*Effect of a Single Injection of Parathyroid Extract*

The dosage of parathyroid hormone administered in single injections varied from 10 to 100 (Collip) units per 100 gm. of body weight. The postinjection survivals ranged from 3 to 17 days.

Hormone therapy results in an improvement in the calcification of the incisor. The somewhat wider predentin of the parathyroprivic animals is no longer present. The injection also has a characteristic effect on the dentin (Figs. 1, 2 and 4). The dentin which is forming and calcifying at the time is sometimes sharply demarcated against the preinjection dentin by a fine distinct hematoxylin staining line. The primary effect is a hypocalcified eosin stripe. The secondary effect is a hematoxylin staining zone which extends to the intermediate dentin border. This hematoxylin assumes a characteristic blue color, the intensity of which varies in different animals (Figs. 3, 4 and 6). The total effect also varies in different areas of the incisor. Thus, in the proximal portion, the eosin stripe is wider and the hematoxylin area is narrower. Nevertheless, the primary and secondary effects attain a constant width in the middle third in both the labial and lingual portions of the dentin.

Rats 37 and 38 both received 14.5 units on the 20th day after operation and were killed 3 days after administration of the hormone. Micrometer measurements indicate typical injection zones of 2.9 and 3 days respectively. The primary hypocalcified response however is very weak.

When higher doses of hormone are administered a more prominent reaction is often induced. This is indicated in Rat 29, which received 64.2 units 98 days after operation. The effect was most pronounced on the lingual portion of the upper incisor where a very heavy secondary hematoxylin stripe was noted (Figs. 1, 2 and 4). This zone is  $66\mu$  wide, which indicates 4.1 days of formation. The postinjection survival was 4 days.

Our experiment includes a series of 6 animals surviving the injection for periods longer than 4 days (Table I). In this group the injection effect persists either for the entire survival periods (Fig. 3), or for shorter periods, so that the dentin returns gradually to the condition of disturbed calcification preceding therapy.

In such instances there is a sharp indication of the cessation of the therapeutic effect. In Rat 68, which had a survival period of 17 days, the typical primary and secondary response affected a zone of dentin formed during the first few days, but the dentin formed subsequently showed what appeared to be normal calcification for the remainder of the survival period.

### *Effect of Multiple Injections of Parathyroid Hormone*

Six parathyroidectomized animals were given multiple injections of parathyroid extract, the total dosage ranging from 94 to 274 (Collip) units (Table I). The effect produced was usually the same as that resulting from a single injection, *i.e.* an eosin stripe which constituted the primary response to the first injection, followed by a hematoxylin zone corresponding to the secondary response of the first injection, and the combined effect of the remaining injections.

In Rats 111 and 108 the last injection was administered 7 days before death. The effect is noted to persist until death (Fig. 6). However, in Rat 112, which also belongs to the same group and received a similar dosage, there is a return to the disturbed parathyroprivic condition before death. The fact that the dentin was more severely disturbed following parathyroidectomy in Rat 112 than in the other 2 animals may account for this observation.

Table I shows the close agreement between the calculated survival (last column) and the actual survival after the first injection (column before the last). The calculated survival was obtained by measuring in microns the total width of the primary eosin effect and the secondary hematoxylin effect and dividing the amount by 16. Sixteen microns represents the average daily growth of dentin.

In 16 animals of the group treated with parathyroid hormone a definite cytological change was observed in the active ganoblasts which were situated at the level of the middle third of the enamel matrix. At their distal portion adjacent to the enamel matrix, clear globular inclusions were noted (Fig. 5). These were not seen in normal animals treated with parathyroid extract or in animals that were only parathyroidectomized.

Neither an abnormal number of osteoclasts nor osteitis fibrosa was detected in the parathyroprivic rats that were treated with parathyroid extract in the amounts indicated (Table I).

*Replacement Therapy with Effect of Single Administrations of Calciferol (Vitamin D)*

In this series 30 animals were given oral administrations of calciferol in doses ranging from 46,000 to 644,000 international units. The animals were treated at 42 to 159 days following parathyroidectomy (Table II). The postadministration period was from 1 to 10 days. The dentin reaction is similar to that obtained when parathyroid extract is administered (Figs. 8 and 9). Also, in 13 animals of this group the same type of cytological reaction in the ganoblasts as is described above was observed.

In the group that received smaller doses of calciferol (Table II) it was difficult to detect a response in the dentin and no inclusions in the ganoblasts were noted.

Rat 126 received 276,000 international units and was permitted to live 10 days following administration. Histological examination shows that the secondary hypercalcification effect on the dentin persisted for 10 days. Similar persistence of effect was noted in Rat 125 which survived 7 days.

The most delicate reaction was observed in Rats 113 and 116. These animals received respectively 552,000 and 644,000 units of calciferol. They were both killed 1 day after treatment. Careful histological examination showed a narrow hematoxylin staining line next to the pulp with an intervening narrow predentin border (Fig. 7). The position of the experimental hematoxylin border indicates that it was produced at about the time of the treatment. The inclusions in the ganoblasts noted in other animals were also present here.

## DISCUSSION

*Effect of Replacement Therapy with Parathyroid Extract*

The effect of parathyroid extract on parathyroprivic dentin is of the same type as that observed by Schour and Ham,<sup>2</sup> and Schour, Tweedy and McJunkin<sup>3</sup> in normal dentin, namely a short primary hypocalcified reaction and a secondary overcalcified reaction. However, doses (10 Collip units per 100 gm. of weight) which were ineffective in normal animals were sufficient to produce the typical response in the experimental animals and to reduce the predentin width in the parathyroprivic dentin.



The secondary reaction effected an overcalcification or recovery which tended to continue throughout the survival periods that were tested (1-10 days). In some instances of survival of longer than 6 days the deeper staining reaction with hematoxylin receded during the later portion of the survival period and blended into the staining reaction of a normally calcified dentin.

We were able to calculate the time interval that elapsed between the time of the first injection and the death of the animal by measuring the width of the parathyroprivic dentin that was affected by the administration of the parathyroid hormone in  $\mu$  and dividing this amount by 16 (which represents the number of  $\mu$  of dentin per day laid down normally in the incisor of the rat). This estimate was found to correspond to the actual time of survival with an average accuracy of one-half of 1 day. The rate of apposition of the experimental dentin is therefore similar to that of normal dentin. These findings are in disagreement with those of Von Spreter,<sup>1</sup> who reports a rate of apposition of 8 to 12  $\mu$  per 24 hours and who accepts 10  $\mu$  as the normal daily rate of dentin apposition.

The explanation of the primary and secondary reaction might be made tentatively on the basis of the change in concentration of serum calcium. There is the possibility of obtaining a beautiful correlation if the serum calcium and phosphorus can be determined at a number of intervals before death. As in the case of experimental hyperparathyroidism in normal rats,<sup>2, 3</sup> the primary hypocalcified dentin zone is perhaps associated with a period of calcium mobilization which results in an increased calcium level in the blood. The secondary hypercalcification effect is perhaps associated with the lowering of the serum calcium.

Some of the histological data do not support this possible correlation between the histological picture and the chemical reaction. The extent of the hypocalcified stripe usually indicates a shorter chronological duration than the period of the rise in calcium. The width of the primary reaction possibly corresponds more nearly to the period concerned with the initial disturbances incident to the time of active mobilization. It is also possible that the extent of the primary reaction is reduced by the process of secondary calcification that may be especially effective during the period of the decline of serum calcium.

The present as well as previous studies indicate clearly that the

dentin reaction differs from that of bone. Von Spreter<sup>1</sup> was unable to demonstrate osteitis fibrosa and abnormal osteoclastic activity in parathyroprivic animals. Our findings confirm this observation. The absence of the established histological symptoms of osteitis fibrosa in experimental hyperparathyroidism may be attributable to the fact that the dosage of parathyroid extract was insufficient both to compensate for the parathyroprivic conditions and to produce symptoms of overdosage.

*Effect of Calciferol on the Parathyroprivic Dentin: A Comparison with the Effects of Parathyroid Extract*

In our study no differentiation between therapy with calciferol and parathyroid extract was noted; the same primary and secondary reaction in the dentin occurred in the animals injected with parathyroid extract as in the animals treated with calciferol. Table II indicates a correlation between our histological findings following the administration of calciferol and the serum calcium values. The latter appear to be similar although slightly lower than those found in hypervitaminosis D in normal rats.<sup>2</sup>

The portion of the postinjection dentin which shows the primary hypocalcified reaction is as a rule narrower than the portion showing the secondary hematoxylin staining reaction. This is perhaps associated with the fact that the rise of the serum calcium extends over a shorter period of time than its subsequent decline to the original level. The width of the dentin showing the primary reaction is less than the amount of dentin that formed during the duration of the serum calcium rise. Here, as in the case of the injections of parathyroid extract, it is possible that the original width of the dentin involved in the primary response was greater but became partly obliterated by a process of secondary calcification that may have occurred during the duration of the secondary reaction.

The cytological inclusions in the enamel-forming cells suggest an increased sensitivity of these cells to parathyroid extract and calciferol.

The mechanism of action of vitamin D has as yet not been determined. Taylor, Weld, Branion and Kay<sup>6</sup> postulate an action through stimulation of the parathyroids. They report negative results with concentrated vitamin D in attempts to alleviate para-

thyropriva tetany. Success of other workers is attributed to the presence of accessory parathyroid rests which can only be removed by a free dissection of the neck. Jones <sup>7</sup> was unable to raise the serum calcium level in parathyroidectomized dogs with daily doses of 20 cc. of cod liver oil.

Von Spreter <sup>4</sup> does not regard irradiated ergosterol as a substitute for parathyroid extract and suggests the possibility that the irradiated ergosterol exerts its effect through the accessory parathyroids or other organs sensitive to vitamin D, or through compensating endocrine glands.

Hess, Weinstock and Rivkin <sup>8</sup> succeeded in raising the serum calcium of parathyroidectomized animals to non-tetanic levels by using 100 times the therapeutic dose of irradiated ergosterol or more. Shelling,<sup>9</sup> using very large doses of viosterol, was able to raise the calcium level in parathyroidectomized rats from tetanic to hypercalcemic levels, even on diets containing no calcium or no calcium and phosphorus. It has been suggested that the action of vitamin D is to improve calcium absorption. On the basis of Shelling's findings with diets containing no calcium one would be led to conclude that the mobilization of calcium from the bone by vitamin D given in massive doses occurs independently of its therapeutic effect in increasing absorption. At this juncture, it would be well to indicate that much of the confusion about vitamin D action is caused by the failure of investigators to distinguish between the action of therapeutic and massive doses.

Recently, one of us and coworkers <sup>10</sup> have shown that injection of massive doses of calciferol were ineffective in increasing the serum calcium of the nephrectomized rat, which had previously been thyroparathyroidectomized, but were effective in increasing the serum calcium after nephrectomy alone.

We interpret our results to indicate that with the doses of calciferol employed in this study the dentin response in the parathyroidectomized animal is the same as that obtained with parathyroid extract. This finding concurs with that of those who believe massive doses of vitamin D do not necessitate the presence of the parathyroid in an otherwise intact animal in order to exert their effects.

*Duration of the Effect of Single Doses of Parathyroid Extract or Calciferol*

Our findings of prolonged improved effects on the dentin calcification for as long as 10 to 17 days following a single administration suggest the advisability of an experimental study on the duration effects of single administrations of therapeutic doses of calciferol. If single therapeutic doses produce prolonged effects for comparable durations, the danger of overdose by the accumulated effect of daily administrations would have to be considered.

Our findings of a 3 to 4 day duration effect of single administration of 14–20 units of parathyroid extract differ from those of von Spreter<sup>1</sup> who reports for this dosage a maximal duration effect of 24 hours.

*The Calciotraumatic Ring*

The acute reaction that was found to be registered in the calcification of the dentin at the border of the preexperimental and postexperimental dentin in the rats studied in the preceding report<sup>6</sup> was also present in many of the animals in this study (Fig. 7). An examination of the microphotographs published by von Spreter<sup>1</sup> on the effect of injections of parathyroid extract on the parathyroprivic dentin shows the calciotraumatic ring very distinctly. Erdheim's<sup>11</sup> transplantation experiments also show evidences of the calciotraumatic ring.

## SUMMARY AND CONCLUSIONS

This study is based on 59 parathyroidectomized rats that were operated on between the ages of 21 and 93 days. The animals were divided into 2 groups. In Group I, 29 received 1 to 4 injections of parathyroid extract. The total dosage ranged from 10 to 274 (Collip) units. The survival period was from 3 to 17 days. Of Group II, 30 received a single administration of 46,000 to 644,000 international units of calciferol. The survival period was from 1 to 10 days. Serum calcium values were obtained immediately preceding death.

The histological findings in the dentin of the incisors of these animals were similar in Groups I and II and consisted of: (a) a primary response characterized by an eosin staining zone; (b) a secondary response in the form of a hematoxylin staining zone;

(c) in the majority of cases a sharp hematoxylin staining ring at the border of the preexperimental and postexperimental dentin; and (d) cytological changes in the active enamel-forming cells in one-half of the animals.

In Group II the serum calcium was found to rise during the first 3 postadministration days and then to decline more slowly to the original level. The level and rapidity of the rise increased with the dosage. The serum calcium values were lower than those found in corresponding conditions in the unoperated animals. The histological effect of single doses lasted as long as 10 days in animals treated with parathyroid extract and calciferol. In 2 rats treated with parathyroid extract the effect lasted 17 days.

Our findings confirm previous data that massive doses of calciferol do not necessitate the presence of the parathyroids in order to exert their effects.

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## DESCRIPTION OF PLATE

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### PLATE 144

- Fig. 1. Microphotograph of a longitudinal section of the upper left incisor of Rat 29 which lived 102 days after parathyroidectomy and was given 1 injection of 25 (Collip) units of parathyroid extract per 100 gm. of body weight 4 days before death. The tooth outline is slightly irregular. Note the vascular inclusions and the irregular stratification of the dentin. The injection effect is found near the pulp and is more prominent in the lingual dentin. Al. b. = alveolar bone; En. sp. = enamel space left by the decalcification process of enamel; L. a. p. = labial alveolar periosteum; Od. = odontoblasts. Compare with Figures 2 and 4.  $\times 5.25$ .
- Fig. 2. Microphotograph of the proximal third of the lingual dentin indicated in Figure 1. D. po. = dentin laid down previous to replacement therapy; D' = dentin laid down and calcified after the injection showing modified and improved calcification. This dentin (D') is  $66\ \mu$  wide, corresponding with the 4 day survival period after the injection ( $4 \times 16$ ). Cem. = cementum; Od. = odontoblasts; P. d. m. = periodontal membrane; Pre. = predentin.  $\times 45.5$ .
- Fig. 3. Microphotograph of a longitudinal section from the labial dentin of the upper right incisor of Rat 67 which lived 39 days after parathyroidectomy and was given 1 injection of 78 (Collip) units of parathyroid extract 9 days before death. Note the characteristic parathyroprivic dentin (D. po.) and the more normal calcification following replacement therapy. En. sp. = enamel space; D. m. = mantle dentin; D' = dentin formed and calcified after injection; Pre. = predentin; Od. = odontoblasts.  $\times 120$ .
- Fig. 4. Microphotograph from the middle third of the labial dentin indicated in Figure 1. L. a. p. = labial alveolar periosteum; En. ep. = enamel epithelium. Note its disturbance at X. En. sp. = enamel space showing persistence of organic enamel matrix. D. po. = dentin laid down before replacement therapy showing interglobular dentin and indications of vascular inclusions; D' = dentin laid down and calcified after the injection showing modified calcification and  $65\ \mu$  wide. This amount corresponds closely to the survival period after the injection; Od. = odontoblasts; Pre. = predentin.  $\times 45.5$ .

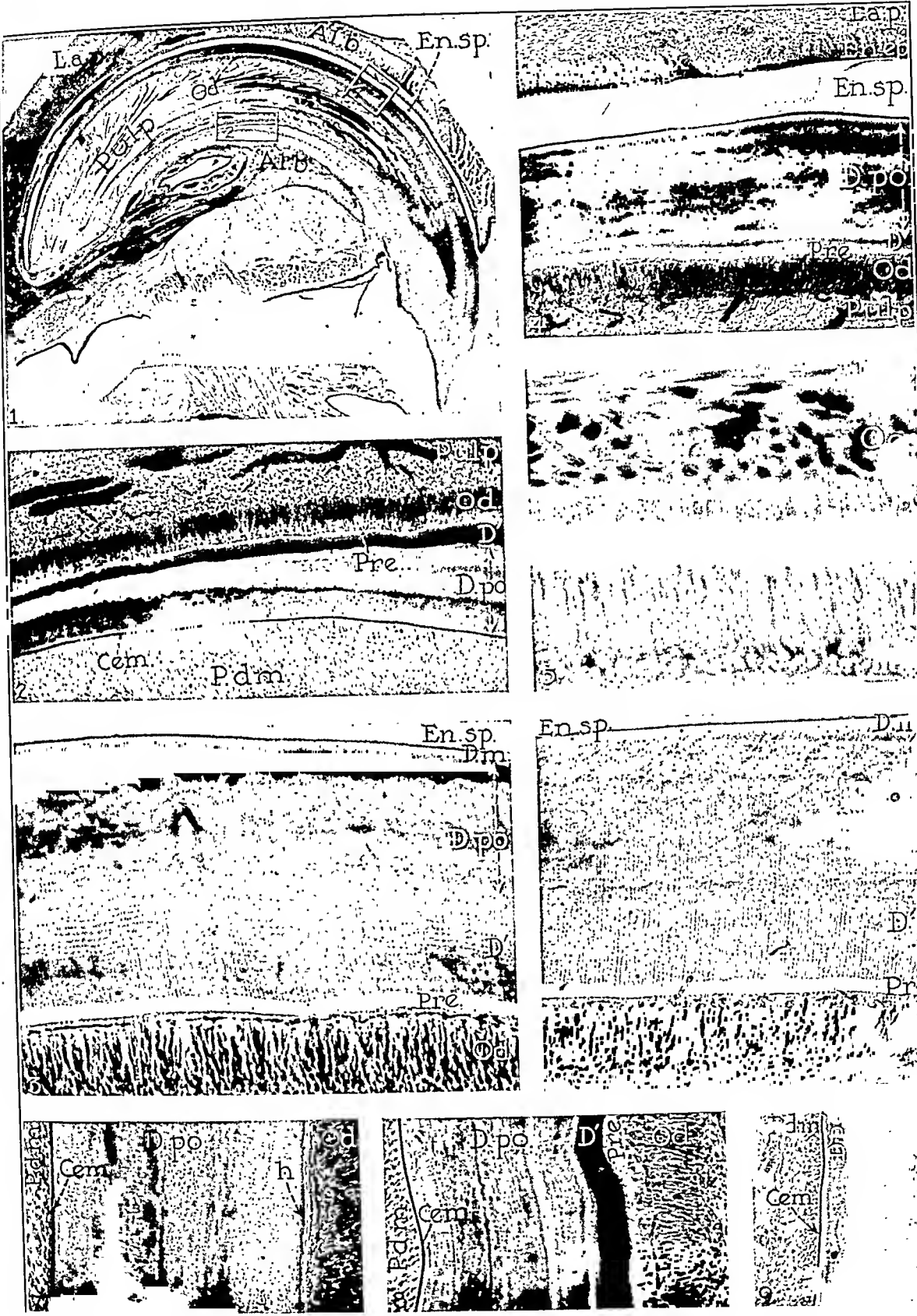
Fig. 5. Microphotograph of a longitudinal section from the proximal enamel epithelium of the upper incisor of Rat 38 which lived 20 days after parathyroidectomy and was given 1 injection of 14.7 (Collip) units of parathyroid extract 3 days before death. Note the globules at the distal end of the ganoblasts. Gan. = ganoblasts; L. a. p. = labial alveolar periosteum; O. e. p. = outer enamel epithelium.  $\times 420$ .

Fig. 6. Microphotograph of a longitudinal section from the midregion of the labial dentin of the upper left incisor of Rat 111 which lived 146 days after parathyroidectomy. It received 2 injections of parathyroid extract 3 days apart. The last injection was given 7 days before death. D. po. = dentin laid down previous to replacement therapy; D' = dentin laid down and calcified after the injection. This dentin shows a more homogeneous calcification. En. sp. = enamel space; D. m. = mantle dentin; Od. = odontoblasts; Pre. = predentin.  $\times 100$ .

Fig. 7. Microphotograph of a longitudinal section from the midregion of the lingual dentin of the lower right incisor of Rat 113 which lived 121 days after parathyroidectomy and was given 1 administration of 552,000 international units of calciferol 24 hours before death. D. po. = dentin laid down previous to the administration. Note the alternate zones of various degrees of calcification disturbances, particularly the uncalcified zone formed preceding the time of administration. Note the very narrow hematoxylin staining line (h) immediately next to the narrow predentin. This line was produced by the administration of calciferol and corresponds in position to the time of administration of the calciferol. Cem. = cementum; Od. = odontoblasts; P. d. m. = periodontal membrane.  $\times 100$ .

Fig. 8. Microphotograph of a longitudinal section from the midregion of the lingual dentin of the upper incisor of Rat 17A which lived 60 days after parathyroidectomy and was given 184,000 international units of calciferol 96 hours before death. D. po. = dentin laid down before administration of calciferol. Note its alternate zones of varying degree of disturbances in calcification. D' = dentin laid down and calcified after the administration. This dentin is  $68\ \mu$  wide, corresponding with the 96 hour survival period after the administration of calciferol. Cem. = cementum; Od. = odontoblasts; P. d. m. = periodontal membrane; Pre. = predentin.  $\times 87.5$ .

Fig. 9. Microphotograph of a longitudinal section from the proximal third of the lingual dentin of the upper left incisor of Rat 6A which lived 62 days after parathyroidectomy and was given 460,000 international units of calciferol 144 hours before death. D. po. = dentin laid down previous to the administration. D' = dentin laid down and calcified after the administration of calciferol. This dentin is  $103\ \mu$  wide and corresponds closely to the 144 hour survival period after the administration. Cem. = cementum; Od. = odontoblasts; P. d. m. = periodontal membrane; Pre. = predentin.  $\times 44.5$ .







MORPHOLOGICAL CHANGES IN THE PITUITARIES OF RATS  
RESULTING FROM COMBINED THYROIDECTOMY  
AND GONADECTOMY\*

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The recent physiological developments in our knowledge of the reciprocal relations between the pituitary and other endocrine organs need correlation with the morphological changes associated with altered secretory phenomena. Just as the many studies made of the primary lesions of the pituitary affecting other organs of internal secretion prove to be of great practical and clinical importance, so do changes reflected in the pituitary when the peripheral endocrine organs have been altered furnish data of theoretical significance.

"Castration cells" which develop in the pituitary of the rat after removal of the gonads are well known. Less well known are the "thyroidectomy cells" which appear in the pituitaries of all animals. As some of the literature on the nature of these cells has been reviewed in a recent study of such cells,<sup>1</sup> it need not be reviewed here. There has been considerable difference of opinion as to whether these cells are chromophobes or basophiles, and whether they are identical with castration cells or not. In a study of 9 male thyroidectomized rats Sevringhaus and coworkers<sup>2</sup> report that after thyroidectomy in the rat the basophile increase gives "to the pituitary the castrate appearance," that "large numbers of typical castration cells are present," and that "basophiles of the thyroidectomized rats are similar to those of castrate and thyroid-treated rats."

It was thought that a study of the pituitary after thyroidectomy and gonadectomy had been performed at the same time would be a means of determining whether or not thyroidectomy cells are identical with castration cells. The results obtained would have a functional significance, which will be discussed later. In previous reports<sup>3, 4</sup> it was stated that thyroidectomy cells can be distinguished from castration cells, but histological descriptions of changes in the pituitary following combined thyroidectomy and

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gonadectomy have not been published by the author. These changes were described orally, however, at the meeting of the American Association of Pathologists and Bacteriologists in 1936.

### METHODS

The pituitaries of 32 male and 30 female white rats which were thyroidectomized and gonadectomized at the same time (35 to 40 days of age) were studied. The rats were killed from 44 to 171 days after operation. In some animals one adrenal was removed as well. The pituitaries and other organs were weighed and then fixed in Helly's fluid and stained with Mallory's aniline blue stain for connective tissue.

### RESULTS

*Histological Changes:* The pituitaries of rats that had been submitted to this combined operation were compared with those of over 200 rats that had been merely thyroidectomized, and with those of 29 rats that had been merely castrated. On each microscopic slide were mounted sections of several pituitaries of rats subjected to different types of operations, so that the staining technique would be the same for the group. This procedure makes patent the difference in staining properties between thyroidectomy and castration cells. Furthermore, in the pituitaries of rats with combined operations the distinction is just as clear. The neglect to control staining by mounting on the same slide experimental and control pituitaries probably accounts for many of the discrepancies found in the literature.

In the pituitaries from rats that had a combined operation there are sometimes considerably more thyroidectomy cells than castration cells. The castration cells show deep blue staining coarse granules, whereas the thyroidectomy cells show very fine granules of the size of those seen in chief cells. The hyaline material in the castration cells is usually clearly demarcated from the cell granules, giving a signet ring appearance; whereas the hyaline material in the thyroidectomy cells appears denser in character and involves the entire cell. The thyroidectomy granules are interspersed through the hyalin in several portions of the cell while one or more portions of the cells are free of granules. The junction, therefore, of hyalin and cell granules is a very irregular line.

After thyroidectomy alone, the loss of acidophiles is striking. In certain pituitaries almost every acidophile has disappeared, in others less extensive loss has occurred but conspicuous degranulation of acidophiles is seen. This loss of acidophiles is associated with visceral dwarfing. By looking at the pituitary microscopically one can estimate roughly the degree of retardation of kidney growth, which is corroborated by the weights recorded in the protocols. This is consistent with the generally accepted view that acidophiles elaborate growth hormone, and seems to me to account for the dwarfing of cretins. These experiments have dealt chiefly with thyroidectomy at an early age, but in the few large rats that were thyroidectomized the kidney weights were less than in the litter-mate control, as though even in the adult, maintenance of visceral weights is dependent on acidophiles. The production of thyroidectomy cells seems to occur at the expense of the acidophiles, which are seen in various stages of degranulation, while castration cells are produced without making any demands on the acidophiles. It is as though the cells had a different source and development.

In the earlier experiments dealing with simple thyroidectomy, the thyroidectomy cells seemed definitely basophilic cells, though staining with more difficulty than ordinary basophiles. Since studying pituitaries of rats that had combined operations, the staining properties of the cells could be contrasted with greater detail. In these the impression was gained that the blueness of the thyroidectomy cells was due more to the intracellular hyaline material which forms a diffuse background in which the cell granules appear suspended than to the staining properties of the granules themselves. This may account for the difference of opinion as to whether the thyroidectomy cells are basophiles or chief cells. It may also explain why the descriptions of pituitaries from human cretins and thyroidectomized rabbits do not seem consistent with the findings in the pituitaries of thyroidectomized rats, cats and dogs. For instance, Bryant,<sup>5</sup> and Marine, Rosen and Spark,<sup>6</sup> do not describe hyalin-containing basophilic cells in the thyroidectomized rabbit pituitary, but rather a hypertrophy of chief cells associated with the loss of acidophiles. In a thyroidectomized rabbit pituitary I have studied, cells of the same character as the thyroidectomy cells of rats, cats and dogs have been found, except

that the intracellular hyalin was small in amount and stained inconspicuously so that the blueness of the cell was merely a tinging and not so apparent.\* There may then be species differences in the formation of the hyaline material and it may be that the staining of the hyalin contributes more to the blueness of the cell than does the staining of the granules. These cells are abnormal cells whatever their classification may be.

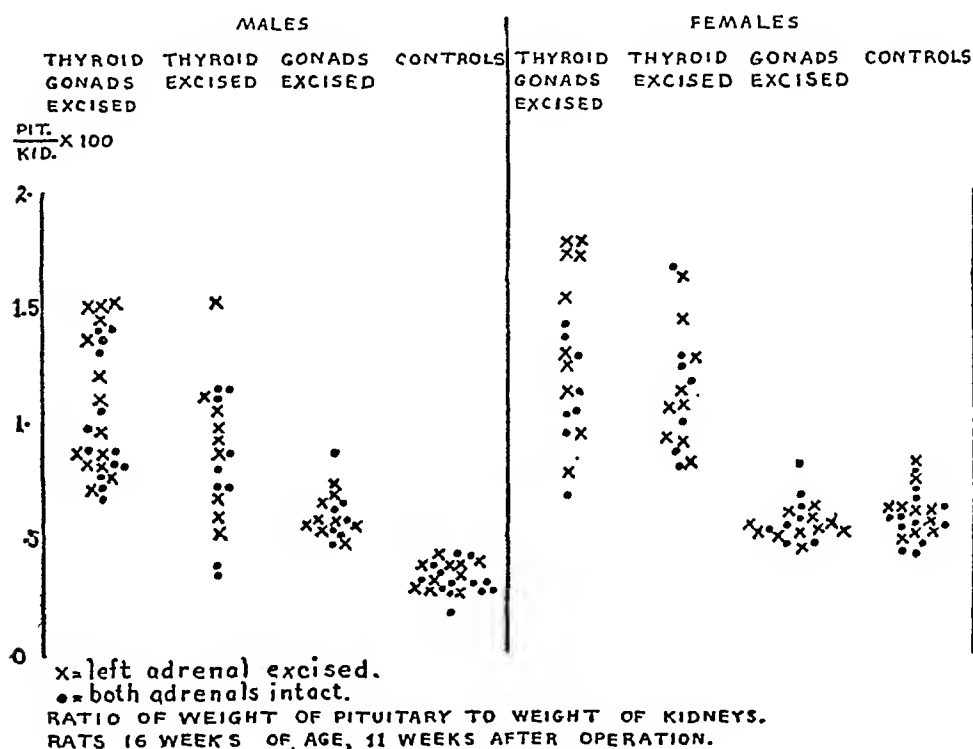


CHART I

The pituitaries of rats after combined operations leave no doubt that the thyroidectomy cells are totally different from castration cells, whether or not their blueness should permit them to be called basophiles.

No recognized change was produced by unilateral adrenalectomy.

*Pituitary Weights:* The weights of rats' pituitaries after thyroidectomy, gonadectomy, and combined thyroidectomy and gonadectomy, were compared in a larger series of rats than those included in the histological study. The highest absolute weights

\* With hematoxylin and eosin staining the peculiar character of thyroidectomy cells cannot be recognized in any species. These cells require special staining.

were obtained in the combined group notwithstanding the marked dwarfing of the animals. In spite of the loss of acidophiles, the increased numbers of abnormal cells and intracellular hyalin had greatly increased the size and weight of the pituitary. Grossly the pituitary was tense, rounded and engorged, in contrast with the pale, flat, flaccid pituitary of normal rats. In Chart 1 only rats that were operated on at 5 weeks and killed at 11 weeks of age are recorded. Unilateral adrenalectomy did not alter the weight. The use of absolute weights in comparing the pituitaries of animals of greatly varying size is confusing. The percentage of pituitary weight to body weight is fallacious because the somatic subcutane-

TABLE I

	Control	Gonadectomized	Thyroidectomized	Thyroidectomized and gonadectomized
Males	6.3 (mean of 23 rats)	10.7 (mean of 16 rats)	9.3 (mean of 18 rats)	13.0 (mean of 27 rats)
Females	9.7 (mean of 22 rats)	8.0 (mean of 24 rats)	12.3 (mean of 16 rats)	13.5 (mean of 18 rats)

ous tissues are greatly thickened in both the stunted thyroidectomized rat and the overly large castrated rat. Therefore, the ratio of pituitary to kidney weight is used in the chart in order to contrast pituitary growth with visceral growth. In terms of absolute weights, the mean weights of pituitaries are shown in Table I.

### DISCUSSION

Considering the fact that there are many secretory principles elaborated by the pituitary and yet only three recognized histological types of cells, several explanations may be considered as possibilities. A cell of given accepted histological type may be secreting more than one hormone, or a given activator principle may have several effects, depending on the peripheral endocrine organ, or we are not recognizing all the histological types of pituitary cells that really exist.

When a peripheral endocrine organ is diseased or ablated, presumably the pituitary cell that reacts in consequence is the cell producing the hormone that affects the peripheral end organ in question. For instance, after castration of rats, certain basophiles seem to be storing within their cytoplasm an excess of secretion,

and it has been shown that such castration pituitaries contain an excess of gonadotropic hormone (Evans and Simpson <sup>7</sup>) and they are discharging into the blood stream an increased amount of gonadotropic hormone. A reasonable explanation for this is that when the internal secretion produced by the peripheral end organ no longer is present in usual amounts, there is compensatory hyperactivity of the pituitary in forming the hormone which stimulates that peripheral endocrine organ. But when there is no end organ to act on, the pituitary secretion which ordinarily stimulates that peripheral organ is accumulating unused in the cell. Similarly, after thyroidectomy certain cells show accumulation of hyaline material which has the appearance of stored secretion, and the pituitary from stunted thyroidectomized rats contains an abundance of thyrotropic hormone.<sup>8</sup> The thyroidectomy cells probably are the cells producing the thyrotropic hormone. Since the present experiments indicate that thyroidectomy cells are distinct from castration cells, these two histological types of cells probably produce different hormones.

In the normal pituitary, basophiles vary in their degree of staining and in the size of their granules. This is generally interpreted as corresponding to phases of secretion and discharge of secretion, the coarsely granular being regarded as "ripe." It is altogether possible, however, that these different blue staining cells really represent cells producing different hormones. That is, that under normal conditions we are not capable of differentiating different types of basophiles, but when the pituitary is altered by thyroidectomy, gonadectomy, or combined thyroidectomy and gonadectomy, then these blue staining cells are dissociated and their histological differences are appreciated.

#### SUMMARY

When thyroidectomy and gonadectomy are carried out at the same time in rats, greater hypertrophy of the pituitary occurs than after thyroidectomy alone or after gonadectomy alone.

After the combined operation both thyroidectomy cells and castration cells appear in the same pituitary.

Thyroidectomy cells are distinctly different from castration cells in many histological characteristics, and the two cells probably produce different hormones.

After the combined operation acidophiles degranulate and largely disappear, just as after thyroidectomy alone. The loss of acidophiles is associated with retardation in visceral growth.

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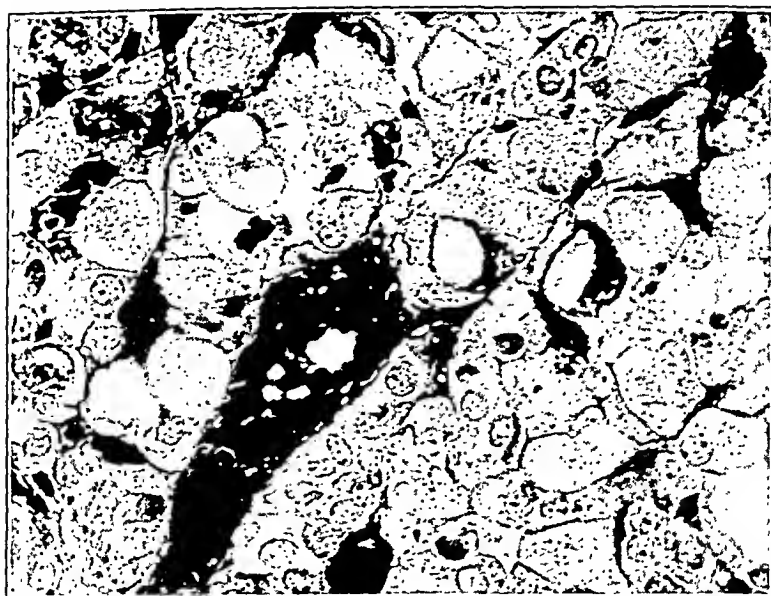


## DESCRIPTION OF PLATE

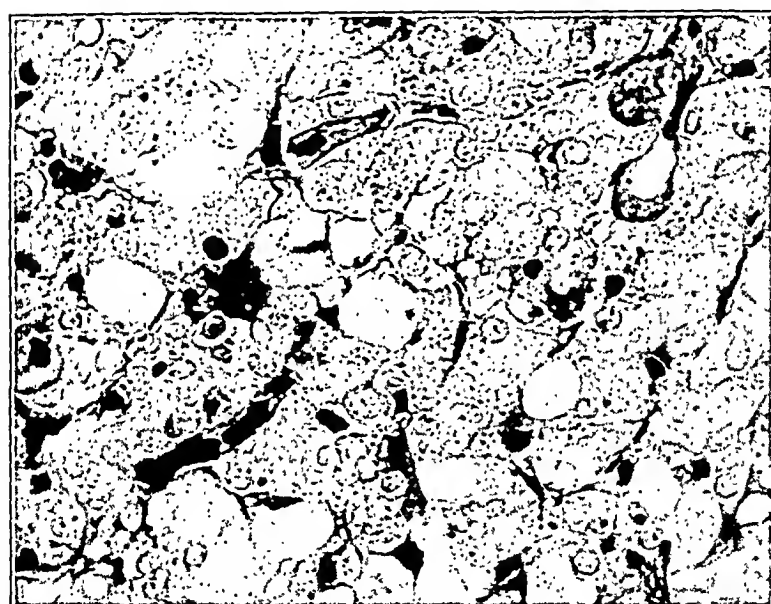
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### PLATE 145

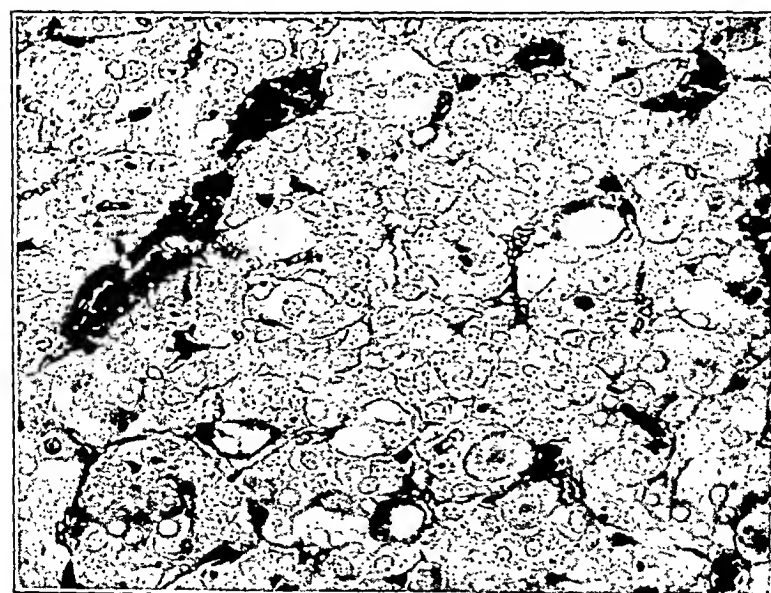
- FIG. 1. Rat 9-D-4, female, 16 weeks of age, 11 weeks after thyroidectomy, gonadectomy, and unilateral adrenalectomy. Two castration cells are seen to the right of the upper end of the large blood vessel. All other hyalin-containing cells are thyroidectomy cells.  $\times 566$ .
- FIG. 2. Rat 9-E-1, male, 16 weeks of age, 11 weeks after thyroidectomy and gonadectomy. Two castration cells are seen in the upper right hand corner. All other hyalin-containing cells in this field are thyroidectomy cells.  $\times 566$ .
- FIG. 3. Rat 10-A-4, male, 16 weeks of age, 11 weeks after thyroidectomy, gonadectomy, and unilateral adrenalectomy. A castration cell is seen in the middle of the lower border, and one at the right a little above the middle. Nearly all the other hyalin-containing cells are thyroidectomy cells.  $\times 393$ .



1



2



3



## SILVER IMPREGNATION OF RETICULUM IN PARAFFIN SECTIONS \*

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Since Maresch first used the Bielschowsky silver stain for the demonstration of connective tissue fibrils, and especially since its results in paraffin sections proved to be at least as reliable if not better than those obtainable with frozen material, the Bielschowsky-Maresch silver impregnation has become one of the most widely used special staining methods. Although many connective tissue stains of different types have been devised since, silver impregnation still ranks first because of its sharp delineation of the finest fibrils. Like all important methods it too has many modifications, each claiming some superiority to the original technique. As I was unable to find data in the literature concerning the relative merits and reliability of the various modifications, and, secondly, as the rôle and importance of the individual steps of the impregnation process are almost unknown, I decided to investigate this problem systematically. For the time being I used formalin-fixed material only, embedded in paraffin, or in celloidin-paraffin according to the rapid method of Erös. It is my plan also, however, to extend further my investigations concerning the action of the fixatives. My results to date are as follows: First, I was impressed by the occasional high degree of similarity, almost identity, of the results obtained by methods seemingly most dissimilar. On the other hand, there is no known method, the results of which would be as reliable and constant as those of, for instance, the hematoxylin nuclear stain, all silver methods being liable to yield more or less variable pictures. Even unexplainable complete failures are by no means rare. There is, however, a great difference in the reliability index of the various methods, some of them being notoriously prone to complete failure, and even in the event of success yielding pictures of variable quality; whereas with other methods failure is most exceptional and the results are remarkably uniform. A second essential difference between the various methods lies in the different amount of reticular meshwork demon-

\* Received for publication July 6, 1937.

strated. Some methods almost invariably reveal a decidedly smaller number of fibrils than are demonstrated by other methods. If one happens to be accustomed to use routinely one of the methods demonstrating a relatively sparsely woven reticulum, he may be convinced, by lack of comparison, of the effectiveness of his method and quite unaware of the fact that other methods demonstrate a much richer fibrillar structure than the method he adopted. He probably would be surprised if informed that he never had seen a really complete fibrillar picture.

The successive steps of the silver impregnation method are discussed below and it is hoped that the information given will be of value to the various workers interested in the demonstration and study of reticulum.

1. *Oxidation-Reduction*: Oxidation of the sections with potassium permanganate, followed by reduction with oxalic or hydrobromic acid, or, according to my experience, even better with acid potassium sulphite (so-called metabisulphite), is an essential part of the procedure, greatly reducing the number of failures and ensuring more uniform results. Wilder suggests the use of a 10 per cent phosphomolybdic acid solution instead. I, too, obtained many beautiful preparations with this latter method, but its reliability being decidedly inferior to that of the permanganate treatment, the routine use of the latter is more recommendable. The undesirable action of the permanganate treatment, consisting of loosening of the sections on the slide and often their actual floating away in the further course of impregnation, can easily be obviated by a suitable technique to be described later. According to Foot, the permanganate is liable to impair nuclear staining; therefore he suggests the use of a pyridine-glycerin mixture instead. I have never observed impairment of nuclear staining caused by permanganate treatment. Moreover, I found the effect of the pyridine-glycerin mixture to be much inferior to that of permanganate oxidation and by no means suitable to replace it. The mechanism of action of the permanganate solution is not well understood. That it is not due to mere oxidation has been proved by Foot. He was unable to obtain the same effect with any other oxidizing agent. I repeated his experiments and tested a few additional substances, such as sodium perborate, ammonium persulphate, chromic acid and a compound solution of iodine. The results were

identical. I also concur in Foot's finding that both manganese salts and oxalic acid in themselves are inert.

2. *Sensitization*: I wish to apply this term to any treatment of the section with metal salts or other substances preceding the use of the ammoniacal silver solution. Suitable sensitization also is an important step, causing the fibrillar structure to become more completely visualized. Tannic acid and certain metal salts are sensitizing agents. The tannic acid treatment seems to be inferior to that of the metal salts as it is less reliable and the ground substance is likely to become dark brown, greatly impairing the clarity of the picture. Hence, metal salts are to be preferred. I assayed the salts of the following metals: aluminum, silver (used in the original method of Bielschowsky), gold, cadmium, chromium, cobalt, copper, iron (ferrous and ferric), mercury, magnesium, manganese, nickel, lead, stibium, tin, uranium (suggested by Wilder) and zinc. Marked and uniform sensitization was obtained with silver, gold, cadmium, ferric, lead, tin and uranium salts. The richest reticulum is obtained with iron sensitization. The sensitizing action of the other metals is either nil, slight, inconstant, or not selective. Gold, lead and tin salts often cause undesirable precipitates, though otherwise the reticular structure is excellent. There remain silver, cadmium, ferric and uranium compounds. As mentioned, silver sensitization is a part of the original Bielschowsky method and of certain of its modifications. Originally the use of a 2 per cent solution of silver nitrate for 24 to 48 hours was recommended. I found that exactly the same effect can be obtained within 2 minutes if a 10 per cent solution is used. In this way time can be saved. The original method warns against a more than superficial rinsing of the sections in distilled water after sensitization, as longer rinsing is liable to weaken the stain. This I cannot confirm. On the contrary, I strongly advise thorough washing of the sections in several changes of distilled water. This slight modification will result in distinctly clearer pictures, better nuclear staining, and complete absence of precipitates. The same technique applies to all other metal sensitizations (cadmium, uranium, and especially iron). I used the nitrates of cadmium and uranium in 1 to 2 per cent solutions. The duration of sensitization is about 1 minute; longer exposure does not enhance the effect nor does the combined use of several metal salts. The ferric com-

pound I used was iron ammonium sulphate, the same substance used in Heidenhain's iron hematoxylin stain. I employed freshly prepared 1 to 2 per cent solutions. The time of exposure should be 1 minute. The peculiarity of iron sensitization is the brilliant metachromasia obtained on gold toning.

3. *Silver Impregnation*: Most modifications concern the preparation of the ammoniacal silver solution. In general, three different types of solutions are used:

1. From silver nitrate silver hydroxide is precipitated with sodium or potassium hydroxide and the precipitate is dissolved in ammonia.

2. From silver nitrate silver carbonate is precipitated by some soluble carbonate and the precipitate is dissolved in ammonia.

3. To the silver nitrate solution ammonia is added drop by drop, until the precipitate which forms on addition of the first few drops is again dissolved.

There are many formulas for the preparation of the solutions, some of which are characterized by almost extravagant accuracy — for instance, the formulas of Kubie and Davidson. I started my experiments with solutions of the Type 1 (silver-ammonia hydroxide). I varied the amount of added alkali from one-half to three volumes. The only difference I noticed was the proportionately quicker action of the more alkaline solutions. However, the final results obtained with the different solutions were extremely similar, indeed identical to such extent that I would have been unable to distinguish the sections stained with the different solutions had I not marked them beforehand. Therefore, in my opinion, too great accuracy in preparing the ammoniacal silver solution is entirely superfluous. Of course, it is better not to use too strongly alkaline solutions as they are liable to damage the sections. Solutions prepared with one-half to three-fourths equivalent amount of alkali are the most suitable, *i.e.* to one volume of 10 per cent silver nitrate solution one-sixth to one-fourth volume of a 10 per cent solution of potassium hydroxide is added. The same applies to carbonate solutions. The amount of ammonia seems to be more important. According to most formulas even a slight excess of ammonia is likely to produce inferior results, especially if the hydroxide type of solution is used. According to my experience there is a certain optimal amount of ammonia; both more or less will produce unsatisfactory results. If there is an excess

of ammonia the picture will be very sharp and distinct, but a part of the fibers will escape impregnation; whereas if too little ammonia is used the ground substance will be dark and the picture blurred. There are different methods for securing the right amount of ammonia, the simplest of which are the following: to the precipitate ammonia is added drop by drop, while the container is continuously shaken, until the last grains are just dissolved and then either (1) silver nitrate is again added cautiously until it is easily dissolved on stirring the solution, or (2) the vessel containing the solution is placed in hot water until black silver precipitate begins to form on its surface. Solutions prepared in either way can be used for 2 or 3 days if kept in stoppered bottles. The silver precipitate that collects on the bottom of the bottle does not interfere with the staining capacity of the solution. Solutions of the carbonate type and those prepared with ammonia only, keep well for at least 5 to 6 days. Solutions of the hydroxide type are to be diluted with distilled water to twice their volume and used at room temperature. The time of exposure is about 1 to 3 minutes. If cadmium, iron or uranium sensitization is used, 1 minute will suffice and the sections will show almost no change in color; whereas if silver sensitization is used it is better to prolong impregnation to 3 minutes, until the sections become pale tobacco brown. Solutions of Types 1 and 2 stain only at higher temperatures (37 to 50° C.) and should be diluted to 4 to 5 times their volume before use. When comparing the different solutions I found that those of the hydroxide type are the most reliable and yield the most uniform results; whereas with the carbonate solution and with the solution prepared with ammonia only, failures are not uncommon. However, in the case of success the silver carbonate stain excels in producing absolutely even, delicately shaded pictures, free of precipitate. All solutions stain the cells also. At times excellent nuclear staining is obtained, on other occasions the cytoplasm will be stained. Very often different parts of the same section show different cellular staining. The cause of this phenomenon is unknown. In general, with cadmium and uranium sensitization the chromatin pattern is more distinct than if iron or silver is used. After impregnation the sections are washed in distilled water for 5 to 10 seconds. Longer exposure to distilled water weakens the stain.

4. *Reduction*: Formalin is used for this purpose, the concentra-



tion of which within wide limits does not appreciably influence the result. In contrast with the findings of Foot I found the reaction of formalin unimportant. Simple commercial formalin, neutralized, slightly alkalized or acidulated solutions, gave identical results. The duration of reduction should be at least 3 minutes. After reduction the sections are washed in running water.

5. *Gold Toning*: Successful toning produces beautiful shades. The reticulum is dark black, collagen fibers are rose to brick red, nuclei rusty brown to deep red. Unfortunately, it is not always possible to produce this range of shades. The cause of occasional failures is unknown. However, there are several factors decidedly enhancing metachromasia. These are iron sensitization, prolongation of gold toning to at least 10 minutes, and finally the reduction of the toning with oxalic acid (according to Laidlaw), or even better with potassium metabisulphite. The action of the latter compound is instantaneous. By employing this combination failures can be prevented with almost absolute certainty.

6. *Fixation*: Fix in a 1 to 2 per cent solution of sodium thiosulphate (hyposulphite) for 1 minute. Longer fixation will impair the distinctness of the finest fibers. After fixation the sections are thoroughly washed in running water, then treated with 2 changes of alcohol, cleared with xylol and mounted in balsam. Foot suggested counterstaining of the sections with hematoxylin and picro-fuchsin. In my opinion this counterstaining is unnecessary; moreover, the fact that after the van Gieson stain it is often impossible to determine whether certain fibers are stained by fuchsin or by gold, outweighs its possible advantages.

In summarizing my results, I may say that all methods omitting either permanganate oxidation or sensitization, or both, are decidedly unreliable and the reticulum picture they yield is, even in the case of success, incomplete. Far the best sensitizing agent I have tried is iron ammonium sulphate.

I wish now to describe my own modification of the Bielschowsky-Maresch reticulum impregnation which gave complete satisfaction in a series of several hundreds of sections. The only material I had failures with is bone marrow, especially the fatty type, whereas highly cellular marrow, as seen in leukemia and in some cases of pernicious anemia, gave beautiful pictures. The poor impregnability of bone marrow is well known to all who have

tried to study its reticulum by means of silver impregnation, and it is mentioned also by Orsós. After having tried all methods described I am convinced that no method is certain of reliability in this respect.

My modification is as follows:

Run paraffin sections through xylol, then 2 changes of alcohol and wash under the tap.

1. Oxidize with a 0.5 to 1 per cent solution of potassium permanganate for 1 to 2 minutes. Rinse in tap water.

2. Decolorize with a 1 to 3 per cent solution of potassium metabisulphite for 1 minute. Wash under the tap for several minutes.

3. Sensitize in a 2 per cent solution of iron ammonium sulphate (violet crystals) in distilled water for 1 minute. Wash under the tap for a few minutes, then run through 2 changes of distilled water.

4. Impregnate with the following solution for 1 minute:

To a 10 per cent silver nitrate solution add one-sixth to one-fourth its volume of a 10 per cent solution of potassium hydroxide. Add strong ammonia water drop by drop, while shaking the container continuously, until the precipitate is completely dissolved. Add again, cautiously, silver nitrate solution drop by drop until the resulting precipitate easily disappears on shaking the solution. Make up the solution with distilled water to twice its volume. It can be kept in a stoppered bottle for 2 days.

5. Rinse quickly in distilled water for 5 to 10 seconds.

6. Reduce for 3 minutes in commercial formalin diluted with tap water to 5 to 10 times its volume. Wash under the tap for a few minutes.

7. Tone in a 0.1 to 0.2 per cent solution of gold chloride for 10 minutes. Rinse in distilled water.

8. Reduce toning in a 1 to 3 per cent solution of potassium metabisulphite for 1 minute.

9. Fix in a 1 to 2 per cent solution of sodium thiosulphate (hyposulphite) for 1 minute.

Wash under the tap. Run through alcohol of increasing percentages. Clear in xylol and mount in balsam.

As mentioned before, paraffin sections occasionally will float away during impregnation with the strongly alkaline silver solu-

tion. This annoyance can be easily prevented by affixing the sections to the slide with gelatin instead of egg albumin-glycerin. The gelatin must be subsequently hardened by formalin fumes. The method is as follows: Dilute the glycerin-gelatin mixture commonly used for fluid preservation of sections with water or glycerin until it remains fluid at room temperature. Spread a thin layer of this solution on the slide and affix sections. Dry the slides in the incubator at 37° C. in formalin fumes for at least 10 hours. (Pour commercial concentrated formalin into an open Petri dish and place it in the incubator.) The formalin has to be removed from the sections as even traces of it will inhibit impregnation. This is easily accomplished by exposing the slides in a similar manner to the action of ammonia vapor for several hours.

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## DESCRIPTION OF PLATES

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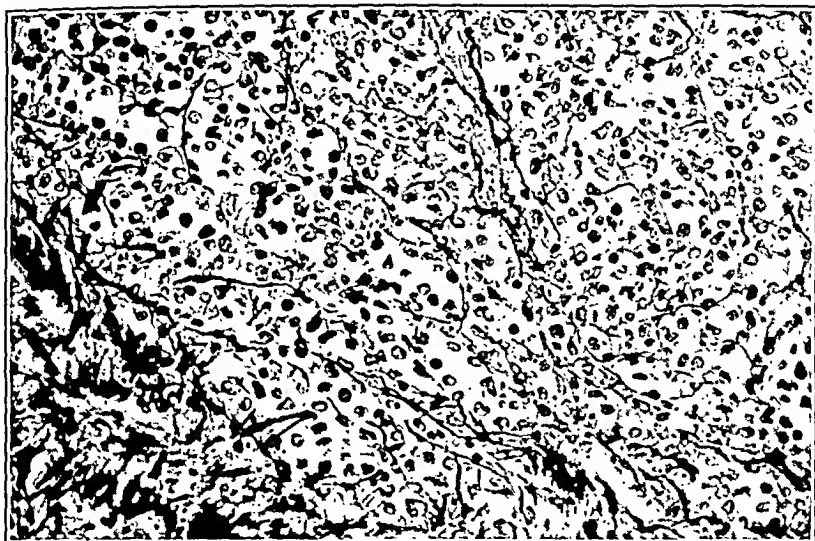
Microphotographs of Figs. 1 to 5 show corresponding fields of serial sections of a block from a case of subcutaneous round cell sarcoma. Figures 6 to 9 show corresponding fields of serial sections from a leukemic spleen. (The distention of the vascular spaces is artificial and was produced by the injection of formalin solution into the splenic vessels.) All microphotographs have been made under strictly identical optical conditions.

### PLATE 146

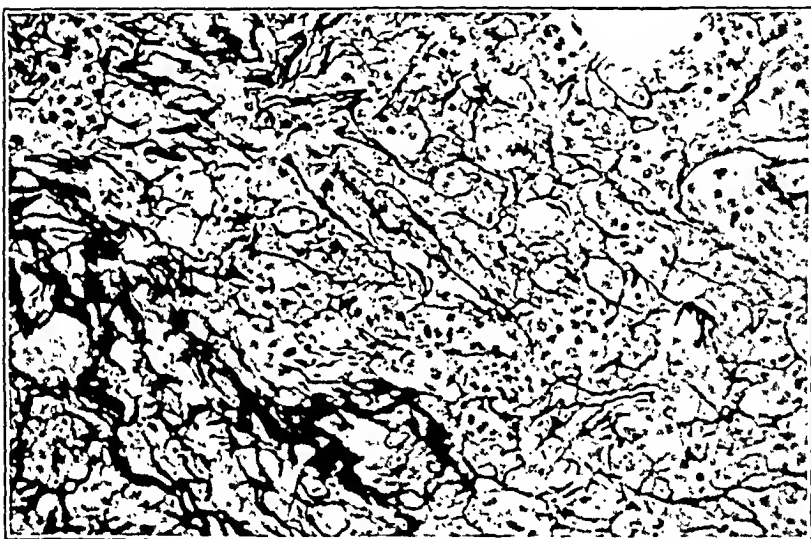
FIG. 1. Foot's stain, Variant II.

FIG. 2. Silver sensitization (author's modification).

FIG. 3. Uranium sensitization (method of Wilder).



1



2



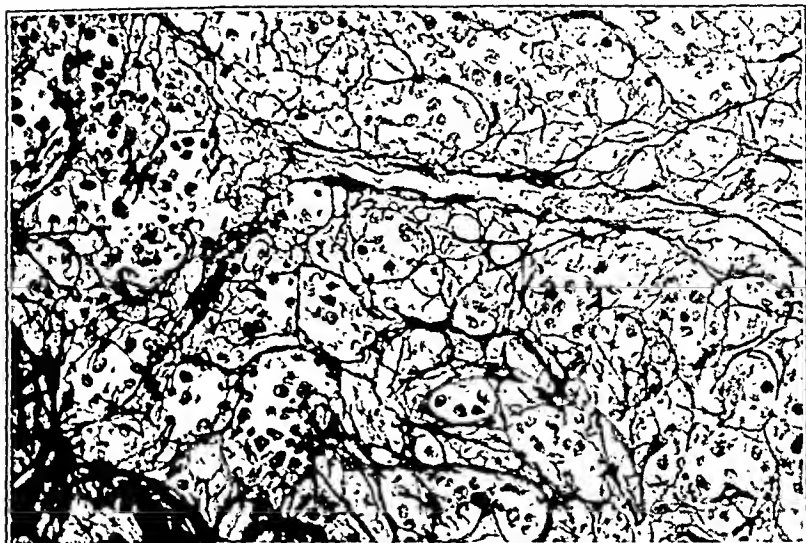
3

PLATE 147

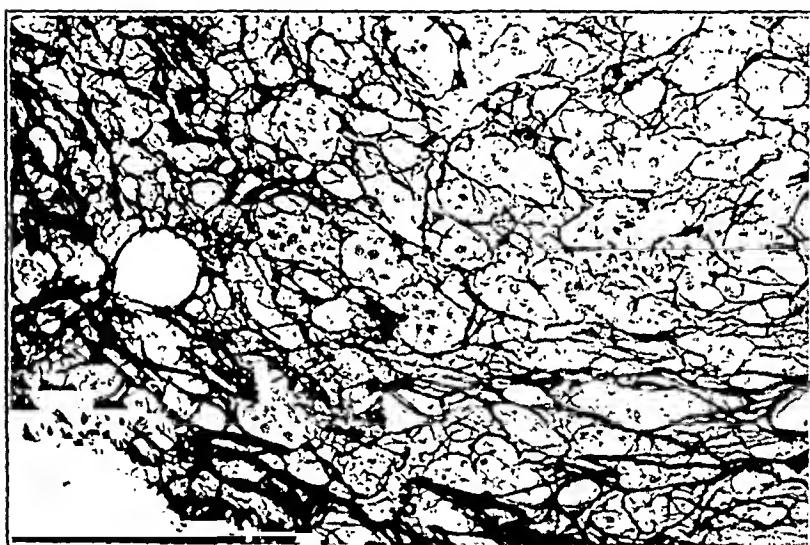
FIG. 4. Cadmium sensitization.

FIG. 5. Iron sensitization.

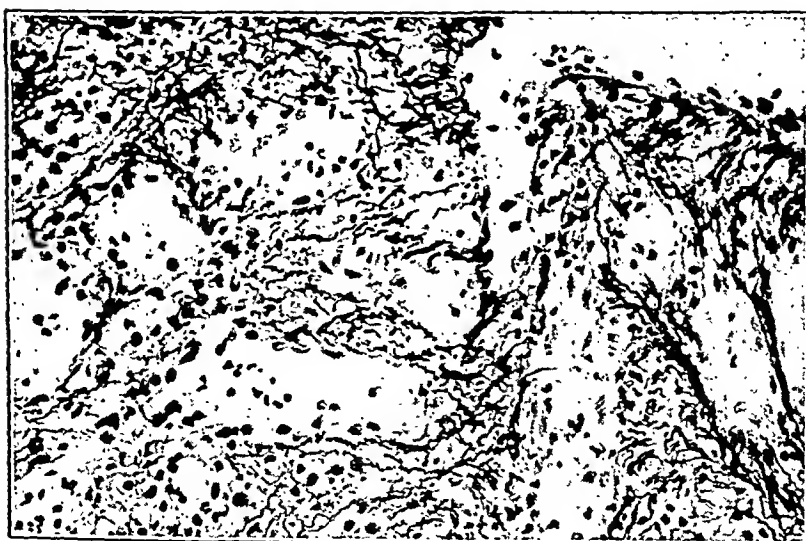
FIG. 6. Foot's stain, Variant II.



4



5



6

Gömöri

Silver Impregnation of Reticulum



PLATE 148

FIG. 7. Uranium sensitization (method of Wilder).

FIG. 8. Cadmium sensitization.

FIG. 9. Iron sensitization.



7



8



9

Gömöri

Silver Impregnation of Reticulum



## CEREBRAL MEDULLOBLASTOMA \*

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The occurrence of primary medulloblastoma in the cerebrum deserves record on account of its rarity. The question has been raised as to whether it exists at all. Therefore, it must be considered whether theories of the origin of medulloblastoma in the cerebellum preclude its occurrence in the cerebrum, and proof must be advanced that the tumor is identical when it arises in the cerebrum.

Bailey and Cushing<sup>1</sup> considered the medulloblast a bipotential cell, which might give rise equally to glial elements and nerve cells. They accepted the hypothesis of Schaper that the cells of the external granular layer, earlier recognized by Hess as a transitory structure, made up a secondary germinal layer and acted as a depot of indifferent constituents able to develop neuroblasts and spongioblasts. They considered medulloblastoma to have its origin in retained rests of embryonic cells of this kind. Accordingly medulloblastomatous tumors might be of a primitive cell type or vary considerably in their growth and degree of departure from the primitive cell form. Thus in his atlas Bailey<sup>2</sup> portrayed medulloblastomas with a preponderance of cells with spherical vesicular nuclei, each with a heavy nucleolus better identified with special silver stains. These he considered neuroblasts. On the other hand, a medulloblastoma might show a fair proportion of spongioblastic elements.

The view of the French school is rather different. To Roussy and Oberling the constituents of the medulloblastoma of Bailey and Cushing are not indifferent cells. They find a preponderance of neuroblastic cells. Considering the similarity of the elements of the embryonic medullary tube, the "neurosponge," they would designate the tumor neurospongioma. They admit that these tumors are not made up of pure growths of a cell type stopped at one precise point in cell evolution. For that reason they would

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reject other terms that have been suggested — neurocytoma, neuroblastoma and neurogliocytoma.

There is much to suggest the origin of medulloblastoma in embryonic rests, apart from the morphological characteristics recalling a type cell found in the embryo. The majority give rise to symptoms early in life, and occur in the midline where cell rests are more frequent. Brody and German<sup>3</sup> found cell rests in the region of the posterior medullary velum in 75 out of 400 supposedly normal cerebellums. Wohlwill<sup>4</sup> noted the roof of the fourth ventricle as a favorite site of rests. Commenting on the persistence of germinal clusters in the first month of extrauterine life, he agreed with Wanke<sup>5</sup> that these cells were related to unripe glial elements and neuroblasts. He considered some of the cells more mature neuroblasts. The occurrence of medulloblastomas in identical twins observed by Cushing<sup>6</sup> also gives support to the theory of origin in cell rests.

Stevenson and Echlin<sup>7</sup> have recently suggested that medulloblastoma arises from a specific cerebellar structure. They describe a number of cases of tumor apparently arising from the external granular layer of the cerebellum and suggest the name granuloblastoma. Thereby, they wish to designate a tumor of a cell destined to give a special type of nerve cell — the granule cell. They argue that if the basic Schaper theory be true, then a definitely gliomatous tumor might be expected on occasion. With Schaper, Ostertag,<sup>8</sup> Jacob<sup>9, 10</sup> and Hayashi<sup>11</sup> agree that the external granular layer not only gives rise to the broader molecular layer with nerve cells, the elements of the inner granular layer, but is also a new source of quite indifferent cells for the cerebellum as much for the ganglion cells as for the glial elements. They draw attention to the numerous cell elements migrating from the external granular layer to the inner, elements they consider bipotential. While a specific granule cell stain is wanting, there can be no absolute indication that the external layer forms only granule cells. A theory of origin of medulloblastoma from a specific cerebellar structure must exclude the acceptance of cerebral medulloblastomas.

On the other hand, the theory of origin in cell rests may also be applied to a consideration of medulloblastoma of the cerebrum, for embryonic rests are known to occur there. Jacob<sup>9</sup> describes the broadening of the white matter from the 5th month of fetal life.

Everywhere remnants of the intermediate layer appear as connected bands or as cell tubes and islands. Isolated groups of these embryonal cells may persist for some time. Such heterotopias, surviving from the invasive and migratory phase of cerebral cellular development, have been the subject of many reports. Since they often occur in relation to a blood vessel, although eccentric, they have been misinterpreted as encephalitis congenita.<sup>12</sup> Naturally they have been more frequently observed in the brains of premature infants. Ferraro and Barrera<sup>13</sup> described a remarkable case of megalomyelo-encephaly with diffuse medulloblastosis in a child of 12 years. There was evidence of retained embryonic development with the added element of neoplasia. A suggested explanation was that cells which had not reached their ultimate destination in the central nervous system had maintained their embryonic activity; at points in the cerebrum apparently indifferent cells had gone on to form elements of both the spongioblastic and neuroblastic series — a neoplastic process recalling tuberosclerosis. There may be in the cerebrum, therefore, sources of embryonic cells such as those that account for the appearance of medulloblastoma in the cerebellum.

The following 2 cases show that tumors morphologically identical with the infratentorial medulloblastomas occur also in the cerebrum.

### CASE REPORTS

**CASE 1. *Clinical History:*** Male, aged 36 years. For about 1 year following death of the patient's mother from glioblastoma multiforme, the history was one of personality changes. Difficulty in walking and dizziness preceded by 14 days an increasing left hemiplegia with stupor. There was no other complaint and physical examination revealed no abnormalities save the neurological. By roentgenogram the pineal was found displaced posteriorly and to the left, indicating an expanding lesion within the right cerebral hemisphere. Ventriculogram revealed displacement of the lateral and third ventricles to the left; the right temporal horn was not visualized. At operation a large infiltrating mass presented high up in the temporal lobe and a second mass in the posterior part of the frontal lobe. Much tumor was removed but complete eradication was not possible. Small cystic collections of yellow fluid were encountered.

A course of X-ray treatment was given. Power in the left limbs improved for some 6 weeks. With assistance the patient was able to walk. He remained facetious and overactive mentally. Thereafter deterioration was rapid. Homonymous hemianopsia, blurring of disc margins, paresis and hypesthesia of the left side and general hyperreflexia developed. The patient died 12 weeks from the time of operation.

### *Pathological Findings*

Autopsy was limited to the head. On removal the cerebral hemispheres were found asymmetrical, the gyri flattened and sulci narrowed over the convexity, especially on the right side. In the right temporal region was the raised zone of operative trauma. The chiasm, basal ganglia and midbrain were compressed by the grossly enlarged temporal lobe. The rest of the brain stem and the cerebellum showed no abnormalities.

Section showed in the entire right occipital lobe and in the posterior part of the parietal lobe, a granular, cream colored tumor with indefinite edges and yellowish degenerated and hemorrhagic areas. It measured approximately 3 by 3 by 3.5 cm. It extended to the white matter of the right temporal lobe where there was a large cystic zone, the site of operation. The cyst communicated with the inferior horn of the lateral ventricle. It measured 6 cm. in length by 5.5 and 3.5 cm. The ependymal lining of the ventricles was smooth; the right lateral ventricle was narrowed, and the left dilated.

### *Microscopic Examination*

The tumor extensively invaded the central white matter and cortex. It consisted of cells fairly densely packed save in the central areas of necrosis and in the periphery where columns were especially arranged along the blood vessels which were numerous and showed considerable endothelial hyperplasia. A pseudorosette arrangement was sometimes observed, tumor cells being gathered in rings with the tail of pyriform protoplasm directed towards the center. The cells were mostly round, some polygonal and a few more pyriform. The amount of cytoplasm was generally small. Exceptional cells showed a larger amount, but in it no granules, vacuoles, blepharoplasts or Nissl substance were found. The nuclei were round or oval and their chromatin arranged in deeply staining masses. Infrequently a definite nucleolus was found. The variation in the diameter of the nucleus was represented graphically. In general like limits of variation were found in different parts of the tumor. Local collections of larger or small nuclear types were rarely encountered. The impression was that at no special points was there deviation towards a different cell type. Division by mitosis and amitosis was frequent, with preponderance

of the latter method. Around the blood vessels large vacuolated mononuclears were found and in areas of operation and irradiation there was degeneration with the presence of numerous lymphocytes, phagocytic monocytes and considerable budding of the capillaries and endothelial proliferation. There, too, free adventitial cells occurred and required careful differentiation from atypical tumor cells which might possibly be undergoing change to the spongioblastic series. However, with Cajal's stain no true unipolar or bipolar spongioblasts were shown. While demonstrating a few fibrous astrocytes related to the persisting structure of the invaded parenchyma, it failed to reveal in the tumor cells an affinity for gold. In central areas where tumor cells were densely packed and where the misleading appearance of invaded or degenerated brain substance was lacking, cells taking the gold sublimate stain did not occur. To this evidence that the tumor cells were undifferentiated was added a test with Cajal's reduced silver stain for neurofibrils. This failed to show either neurofibril formation even in the more elongated and tail-like types of cytoplasmic process, or the local deposition of silver at one extremity of the cytoplasm (fibrillogenous zone of Held) which has been taken by some authors as evidence of the differentiation of a neuroblast.

Examination of the contralateral hemisphere, the brain stem and cerebellum failed to show any further extension of the tumor.

*CASE 2. Clinical History:* Female, aged 3 years. The child was a full term baby delivered normally. Development appeared usual, the child walking and talking at the average age.

In July, 1933, the patient fell and struck her head. There was no loss of consciousness. In September she had severe frontal headaches, and in November she developed tremor and stiffness in the left leg so that it was usually maintained in the extended position. About the same time the mother noticed some rigidity on lifting the child and since then weakness of the left leg increased and the child became unable to walk. Vomiting occurred occasionally; it was not projectile and occurred after taking food. There was incontinence of urine. Tremor of the right hand at rest was noted a fortnight before admission in May, 1934.

Examination in the Neurological Institute showed moderate rigidity in extension, particularly in the left leg. Significant findings were Magnus-de Kleijn reflexes, hyperreflexia, especially in the lower limbs, absence of abdominal reflexes, dilated pupils, the left failing to react to light, optic atrophy of left disc, thought to be due to preceding papilledema, and blurring of the right disc margins. Plantar responses were not elicited. The cerebrospinal fluid was yellow, with 6 cells, 508 mg. protein, 63 mg. sugar, and 693 mg. chlorides per 100 cc. fluid. The Wassermann reaction on the fluid was negative



and the colloidal gold curve 111000000. Roentgenograph of the skull showed an area of scattered calcification to the right of the midline, measuring 0.5 by 3 cm. on anterior posterior view, and 4.5 by 2 cm. in the lateral. The flecks of calcification formed an arch roughly paralleling the contour of the lateral ventricle. The impression was one of intraventricular tumor or deep parieto-temporal glioma.

Fits with generalized tremors, profuse sweating, rotary nystagmus on looking to the left, and hyperpyrexia preceded death which came 5 days after admission to the hospital.

### *Pathological Findings*

Complete autopsy was performed. The calvarium was thin with some separation of the sutures, the dura tense, the leptomeninges thin and transparent. The brain weighed 1040 gm. The cerebral hemispheres were asymmetrical; flattening of the gyri was general but most marked in the anterior part of the greatly enlarged right temporal lobe. Herniation through the incisura tentorii cerebelli accounted for deep indentation of the hippocampal and fusiform gyri on the right side, and for displacement of the infundibulum, optic chiasm and cerebral peduncles to the left. From the widened posterior end of the sulcus olfactorius protruded a round, cream colored, slightly lobulated mass, 2 cm. in diameter. The floor of the third ventricle was herniated downwards.

Section of the cerebellum showed no abnormality. Section of the brain stem showed concave indentation of the midbrain in the right side, the substantia nigra and red nucleus being especially compressed. The pons was distorted and the floor of the fourth ventricle showed marked granular ependymitis.

The wide extent of the tumor on the right side from frontal to occipital lobes was shown on section of cerebrum. It involved almost all the temporal lobe and the white matter of the frontal, parietal and occipital lobes. It invaded deeply the caudate nucleus, putamen, globus pallidus and thalamus, and it grew down into the orbital gyri. It was roughly 9.5 cm. in length and 5 cm. at its greatest diameter. The margins were indistinct. The tumor was cream colored, firm and granular except in scattered areas of necrosis and hemorrhage. In the parietal region within the tumor was a large cyst with firm but ragged walls. The left lateral ventricle was compressed and like the right and third ventricle showed granular ependymitis with occasional larger nodules representing tumor.

There were no findings of note in the trunk organs.

*Microscopic Examination*

The tumor in the right hemisphere consisted of densely packed cells with numerous blood vessels and rare areas of necrosis. The cells were frequently arranged in clusters and in the pseudorosette formations of Wright. At the periphery, and especially where invading the white matter, they were found in long columns. Even in these areas the polygonal or round shape of the scanty cytoplasm persisted. Where the cells were more discrete and free from compression, the cytoplasm showed a fluffy outline or tail-like process, such as is regularly associated with the cells of cerebellar medulloblastoma. Calcium occurred in small amounts irregularly, free and in relation to the vessel walls.

The spherical or rather oval shape of the nuclei was defined by a distinct and thick nuclear membrane. They stained deeply and showed a coarse chromatin network. Mitoses were numerous. The variation in size of nuclei is shown graphically in Figure 1. The cell bodies did not stain with Mallory's phosphotungstic acid hematoxylin, though rare blue staining fibers were found in the tumor. They most likely represented glial fibers of the invaded brain tissue.

By Cajal's gold sublimate stain a number of hypertrophied fibrous astrocytes were shown in the periphery of the tumor between the tumor cells. They were considered to belong to the invaded parenchyma. No elongated immature cells of the spongioblastic or astroblastic series were revealed, nor were neuroblastic forms detected with a silver neurofibril stain.

The wall of the left lateral ventricle was stripped of ependymal cells and there was proliferation of subependymal astrocytes. A large flat tumor mass of essentially the same type as that in the right cerebral hemisphere represented a tumor implant.

Section of midbrain showed marked compression of the substantia nigra, cerebral peduncles and, to a lesser extent, the red nuclei. Ependymal granulations in the wall of the aqueduct of Sylvius partially occluded the lumen at one point. Gliosis and thickening of the external glial membrane were present. The cerebellum and roof of the fourth ventricle were free of tumor growth.

## DISCUSSION

The diagnosis of medulloblastoma in both cases rests on the general features of the cells, round, oval or pyriform with scanty

cytoplasm and well stained nucleus, their fairly uniform size and their arrangement in compact masses, sometimes with pseudo-rosette formation. The perinuclear halo and polygonal shape of the cells of oligodendroglioma were absent. Special stains for oligodendroglia with silver failed but while they are notoriously difficult in tumor tissue, further proof that oligodendroglial cells were not the constituent elements came from Mallory's connective tissue stain. Even in parts taken from the central portions of the tumor there was no suggestion of the blue staining of the inter-

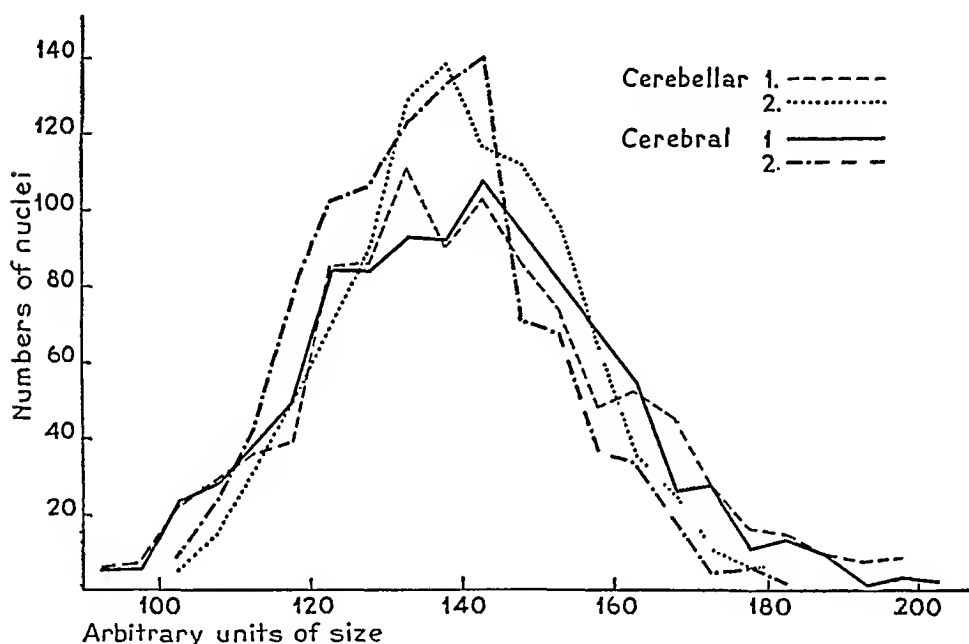


CHART 1

cellular substance such as is found in oligodendroglioma.<sup>14</sup> The tumor cells were unlike those of ependymoma. The absence of true rosettes and the failure of Bailey's neutral ethyl violet-orange G to show blepharoplasts also make the diagnosis of ependymoma unlikely.

With phosphotungstic acid hematoxylin and Cajal's gold sublimate, the tumors were shown not to contain spongioblasts or astrocytes and, therefore, cannot be classified with the more mature gliomas. On the other hand, the proof of a neuroblastic evolution is lacking since neurofibril formation was not detected. Thus the diagnosis of a more mature neuroblastic tumor is eliminated.

The cells of these tumors are then essentially of a younger type and without the staining qualities that would indicate differenti-

ation along neuroblastic or spongioblastic lines. In this respect they are similar to cerebellar medulloblastoma of primitive type.

If the origin of cerebral medulloblastoma is comparable with that of the cerebellar, then the tumor might be expected to occur in the sites of election of congenital rests. Amolsch<sup>12</sup> has found that such points are in the angle between the caudate nucleus and

TABLE I

	1	2	3	4
	Cerebral 1	Cerebral 2	Cerebellar 1	Cerebellar 2
Mean nuclear diameter				
in $\mu$	5.32	5.17	5.30	5.25
S.D.	0.94	0.76	0.42	1.37
P.E.	0.63	0.59	0.28	0.87

the thalamus and at the upper lateral angle of the lateral ventricle in sections through the anterior half of the brain. At term, neurogenic activity is also seen in a few small round cells in the lateral wall of the posterior horn of the ventricle. Owing to the extensive spread in the cases here described it is impossible to determine the point of origin of the tumor.

The similarity of the cerebral to the cerebellar medulloblastomas is also seen in the distribution of nuclear sizes figured in Chart 1. Micrometer measurements of 1000 nuclear diameters were

TABLE II

	1 & 2	1 & 3	1 & 4	2 & 3	2 & 4	3 & 4
Mean Diff.						
P.E. Diff.	0.29	0.30	0.10	0.05	0.09	0.06

made in each of the cerebral tumors and in primitive type cell medulloblastomas of the cerebellum picked at random from the files. The conditions of fixation and staining were strictly similar. From the mean, standard deviation and probable error of these measurements (Table I) the ratio  $\frac{\text{mean difference}}{\text{P. E. difference}}$  was figured (Table II). It gives an index from which one may judge whether the nuclear size distributions are essentially comparable or not.

In the comparison of any two tumors it does not exceed three. The conclusion rests therefore that the constituents of the tumors do not differ in nuclear size significantly.

Since some investigators have described neuroblasts in medulloblastic tumors, our 2 cases were compared with the cerebellar medulloblastomas of primitive type in order to find any differences in this respect. The absence of neurofibril formation has been noted. Other criteria of neuroblastic evolution have been considered. Bailey<sup>2</sup> has suggested that the normal growth of a neuroblast from a less differentiated cell is first shown by swelling of the nucleus. If a similar evolution were taking place in the tumor cells, then possibly the curve of distribution of nuclear sizes in a cerebral medulloblastoma might be biphasic or show a marked shift to the right on comparison with that of medulloblastoma cerebelli of primitive type. No suggestion of this is seen. While the tables serve to indicate that the range of variation is not greater than that which may occur in medulloblastoma cerebelli, that the predominant nuclear sizes of this tumor above and below the cerebrum are the same and the tumors are alike, no further conclusions can be drawn. Heiberg<sup>15</sup> has rightly directed attention to the variation in size and shape of the nucleus in malignant tumors. This consideration must limit the inferences to be drawn from measurements of nuclei in neoplasms.

Conspicuous nucleoli were occasionally found in the cells of both the cerebral and cerebellar medulloblastomas. They have been taken by some authors as a sign of differentiation of a neuroblast. As early as 1896 Pianese<sup>16</sup> noted changes in the nucleolus of cancer cells; today a study of nucleoli is a valuable aid in the diagnosis of malignant tumor, for the nucleolar nucleus ratio is increased considerably. Whether large nucleoli are related to rapid growth or whether the changes are only features of atypical cell proliferation is quite uncertain; yet surely enlargement cannot be taken as an additional diagnostic characteristic of a specific cell type.

It might be suggested that these tumors are made up of neuroblasts which have not yet reached the stage of formation of Nissl substance and neurofibrils. It may be that a number of the cells are of such a type and that primitive cells, sprung from the medullary epithelium and morphologically alike, have determining factors for

one specific evolution. Yet in the absence of characteristics of development of a special type, it is impossible to do more than conjecture in what direction cells of this primitive type are tending. In any case the evidence of evolution is as much lacking for these cerebral as for some cerebellar medulloblastomas. Our studies have shown no essential difference. The histological diagnosis of a primitive cell tumor is also consistent with the life history in the cerebral cases. Cushing has emphasized that it is insufficient to define the microscopic character of a tumor. For each there is a more or less typical life history to be correlated. Here in two cerebral medulloblastomas there is a comparatively rapid course, which contrasts with the history of the majority of Cushing's cases. In 1930 in a review of medulloblastoma Cushing<sup>6</sup> stressed the later age incidence, the slower growth and the more favorable outlook in those of the cerebrum. These features he associated with a more differentiated type of cell. He classified his cases as medulloblastoma, medulloblastoma neuromatosum, and neuroblastoma, according to the degree of deviation from the neuroblastic type. The latter showed least malignant features. As lengthy a preoperative history as 8 years and a 5 year survival after operation were recorded. His cases of tumor of the simple true medulloblastomatous type in the cerebrum were in males aged 37 years and 9 years. They gave histories of 3 years and 4 months and they survived operation for periods of 3 years 4 months and 2 months respectively. In the first of our cases, that of a male, 35 years of age, the history of tumor rapidly proliferating and accounting for death despite operation and irradiation 14 months from onset is without doubt the story of a tumor of a high degree of malignancy. In the second, the early incidence at the age of 3 years and the short course recall typical features of cerebellar medulloblastoma away from the midline. On clinical as well as on histological grounds the diagnosis of undifferentiated cerebral medulloblastoma seems warranted.

#### SUMMARY

The origin of medulloblastoma in the cerebrum is discussed and the 2 cases described are shown to be essentially identical with the cerebellar medulloblastomas in point of morphology and life history.

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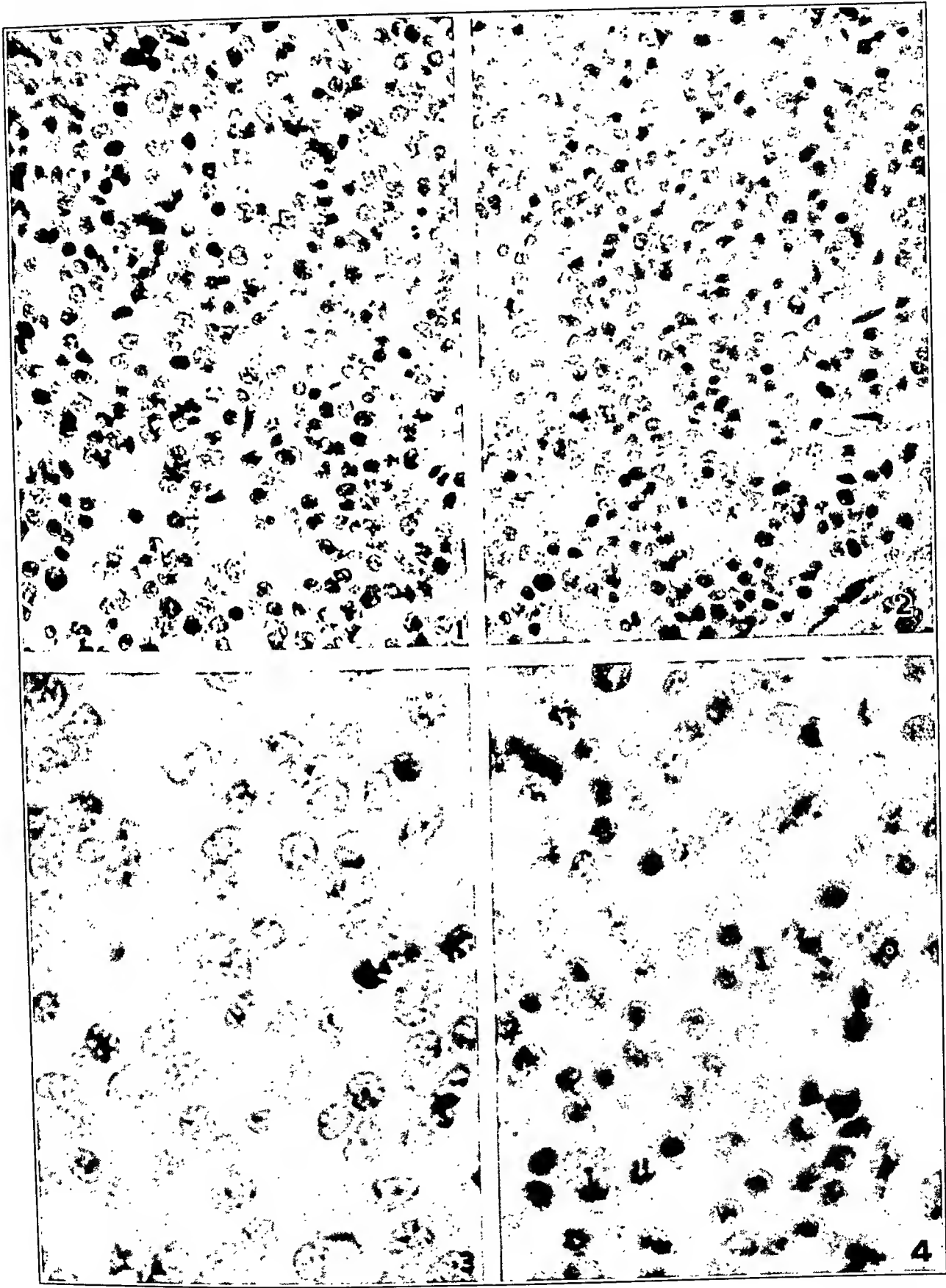


## DESCRIPTION OF PLATE

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### PLATE 149

- FIG. 1. Case 1. Showing uniform distribution of cells and pseudorosettes. Hematoxylin-eosin stain.  $\times 600$ .
- FIG. 2. Case 2. Showing uniform distribution of cells and pseudorosettes. Hematoxylin-eosin stain.  $\times 600$ .
- FIG. 3. Case 1. Cells uniform in size with scanty cytoplasm. Hematoxylin-eosin stain.  $\times 1200$ .
- FIG. 4. Case 2. Showing similar features of cell body and nucleus. Hematoxylin-eosin stain.  $\times 900$ .
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Honeyman

Cerebral Medulloblastoma



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# INDEX OF SUBJECTS

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\*Abstract of paper presented at the meeting of the American Association of Pathologists and Bacteriologists held at Chicago, March 25 and 26, 1937.

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